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THE JOURNAL OF EXPERIMENTAL MEDICINE

ON THE PRESENCE OF CHOLIN AND NEURIN IN THE INTESTINAL CANAL DURING ITS COMPLETE OBSTRUCTION.

A RESEARCH ON AUTOINTOXICATION.

BY BEATTIE NESBITT, M. D.
(From the Pharmacological Laboratory of the Johns Hopkins University.)

In the normal processes of digestion the proteids and carbohydrates of our food are changed into more readily assimilated compounds, which are further altered before reaching the tissues: for example, the peptones,* which, if absorbed unaltered into the system would be very toxic, are changed into nutritive material in passing the intestinal wall. As a result of bacterial activity we may have these compounds broken up in a different manner, giving rise, either as immediate or terminal products of the decomposition of the proteid or carbohydrate molecule, to substances of more or less toxic character. Some of these substances, as phenol, the cresols, the dihydroxy-benzenes, indol and skatol, are known to occur as a result of the constant action of putrefactive bacteria in the large intestine. We may also have a large number of organic acids of the fatty series, as acetic, lactic, butyric, caproic, caprylic, etc., which have been shown to occur in various catarrhal conditions of the intes-

* According to E. Fiquet the poisonous effects usually ascribed to peptones and albumoses are in reality due to ptomaines or other toxins which have not been removed by the ordinary processes of purification. *Compt. rendus Acad. d. sc.*, 1897, p. 1371.

tinal tract, and whose irritating effects have been studied by Bókai.* Gases, as hydrogen sulphide, methylmercaptan,† carbon dioxide, methane and ptomaines, as putrescin (tetramethylendiamin),‡ cadaverin (pentamethylendiamin),‡ ethylidendiamin, are also more or less constantly present.

Opinions vary greatly on the toxicity of these substances, as the pharmacology of but one of them (phenol) has been carefully worked out.

For consideration in this respect they may be divided into three classes: (1) the fatty acids and the various gases, whose action in this connection is almost wholly irritative and need not be considered from the point of view of absorption, except perhaps in the case of infants; (2) substances of the aromatic series, which include phenol, the cresols, the dihydroxy-benzenes, indol and skatol, and (3) the diamins, including putrescin, cadaverin and ethylidendiamin. Substances of the second group are all excreted in the urine as conjugate or ethereal sulphates, and it is by their estimation that we judge of the extent of the putrefactive processes, more especially in the large intestine. From the fact that their molecules are of fairly simple structure, and in the case of most of them completely oxidized, we may consider the amount excreted as a reasonable index of the quantity absorbed.

With phenol (carbolic acid) we are well acquainted; its use as an antiseptic has for years been general, as a poison it has been taken in large quantities, sometimes without fatal results. It has been administered in various diseases, more especially those in which intestinal antisepsis was sought, as typhoid fever, in amount so much larger than the quantity produced in the intestine and absorbed that a comparison would be ridiculous. Thus Brieger § has found the amount excreted in 24 hours by a healthy individual to be about 15 milligrammes, while the textbooks on therapeutics set the maximum daily dose at 600 milligrammes. The cresols or methyl-hydroxy-benzenes are a later addition to the materia medica introduced by Laplace, and have undoubtedly many advantages over carbolic acid. It has been claimed that they are three times less toxic than phenol.‖ I think from my own experiments that

* Bókai, *Arch. f. exp. Pathol. u. Pharmakol*, xxiii, 209; xxiv, 153.

† Nencki, *Jahresb. ü. Thier-Chemie*, xx, 309.

‡ Udránszky and Baumann, *Zeitschr. f. physiol. Chem.*, xiii, 562; xv, 77.
Werigo, Pflüger's *Archiv*, li, 362.
Roos, *Zeitschr. f. physiol. Chem.*, xvi, 192.
Garcia, ibid., xvii, 543.

§ *Zeitschr. f. physiol. Chem.*, ii, 241.

‖ Charteris, *Lancet*, 1894, i, 801.

this figure is too high, but I have found that, while their action on blood-pressure and respiration presents the general phenol picture, recovery is more prompt. The injection of 50 cc. of 0.5 per cent sol. of cresol in normal saline solution into the jugular vein of a dog weighing 4 kilos caused the blood-pressure to fall 41 mm.; it returned to the normal in 13 minutes. Paracresol, according to Baumann and Brieger, occurs in largest amount among the members of this series, all of which give on distillation a reaction with bromine, the ortho- and meta-cresols occurring in traces.

The dihydroxy-benzenes—resorcin, hydroquinon and pyrocatechin—are present in the urine only in traces. All three have been used as medicines, their daily doses being respectively, 0.1-0.6 grammes, 0.25-1.5 grammes, and 0.3-0.2 grammes.

Possibly the substance which is attracting more attention than any of these at the present time is indol, which appears in the urine as indican, or indoxyl potassium sulphate. The amount of indican excreted is for an average man about 12 milligrammes per day; this is equivalent, if all is excreted that is absorbed, to an absorption of 6 milligrammes of indol in 24 hours. Experiments on the toxicity of indol and on its fate in the organism have been made by a number of investigators. Jaffé,* Nencki † and also Baumann ‡ administered considerable quantities of indol to dogs both by subcutaneous injection and by feeding, and although the object of these experiments was to determine the amount of indol that was converted into indican, it was at the same time observed that indol is not a toxic substance. Jaffé found no toxic symptoms following the subcutaneous injection of considerable quantities of indol prepared according to Baeyer's synthesis, and Nencki noted that a dog showed no signs of intoxication after receiving 1 gramme of indol by mouth, but with a dose of 2 grammes during 24 hours developed diarrhœa. Experiments more directly relating to the toxic influence of this substance were next undertaken by Christiani,§ who found that a fowl gave no signs of poisoning when it received 0.07 gramme of indol mixed with bread crumb, but that frogs reacted with decided symptoms in about an hour after the subcutaneous administration of from 1.2-2.4 milligrammes in solutions of 1:1000. The average fatal dose for frogs was 12 mmg. in 1 per cent solution subcutaneously administered. The symptoms were in general like those following the administration of phenol and need

Centralbl. f. d. med. Wiss., 1872, No. 1.

† *Ber. d. deutsch. chem. Gesellsch.*, ix, 299.

‡ Pflüger's *Archiv*, xiii, 285, and *Ber. d. deutsch. chem. Gesellsch.*, ix, 54.

§ *Zeitschr. f. physiol. Chem.*, ii, 273.

not be given in detail. In recent years Rovighi * and Herter † have published more extended researches on the toxicity of indol. Rovighi finds that for rabbits the lethal dose of indol or skatol lies between 1.5 and 2 grammes administered in the course of 24 hours by subcutaneous injection, and that these two products of intestinal putrefaction have a similar physiological action. As summarized in Maly's *Jahresbericht*, the symptoms of poisoning are: torpor, somnolence, widespread paresis, weak action of the heart, reduction of temperature and retention of urine and fæces. The autopsy in cases of acute poisoning showed the portal vessels and the supra-hepatic veins to be highly congested, while in cases of chronic poisoning, especially after the administration of indol, the bile ducts were surrounded with infiltrating small cells, which also filled up the intercellular spaces. The kidneys were congested.

Herter's experiments relate to acute indol poisoning in rabbits and dogs, to chronic indol poisoning in rabbits, and to the effects on man of moderate doses taken by the stomach. As in the experiments of Rovighi, it was found that in acute poisoning with considerable quantities, say 70 cc. of a 0.1 per cent solution of indol injected slowly into the femoral vein of a dog weighing 15 lbs., the symptoms were cardiac and respiratory depression, general prostration, irregular clonic spasms, increased reflex excitability and marked contraction of the pupils. The cause of death appeared to be cardiac rather than respiratory failure. Observations on the temperature and on arterial pressure were not made. Of great interest are Herter's experiments on chronic indol poisoning in rabbits. The daily injection of such small quantities of indol as 10 cc. of a 0.1 per cent solution led to death in the course of 13-22 days. Diminished activity, loss of appetite, profound disturbance of nutrition with marked loss of body weight are the points especially emphasized in this connection. A small ring-tailed monkey was found to be far less susceptible than rabbits, for the monkey received 4 cc. of a 0.1 per cent solution daily for two months without any apparent effect. Highly interesting, too, are Herter's contributions to the study of indol poisoning in man. Three healthy men, varying in age from 25 to 32 years, were induced to take indol during periods of from 6 to 13 days in daily quantities varying in the several subjects from 0.025 to 2 grammes. One of these men, a vigorous medical student, aged 25 years and weighing 160 lbs., consumed no less than 6.8 grammes in divided doses in 6 days, taking on one day

* Abstract in Maly's *Jahresb. u. Thier-Chemie*, xxvi, 456.

† An experimental study of the toxic properties of indol. *New York Med. Journ.*, 1898, July 16 and 23.

as much as 2½ grammes. The first day, after a dose of 1 gramme, no symptoms whatever were noted. Further administration with slightly increased doses led to disturbances of sleep and headache but no distinctly toxic symptoms. Without going further into the details of Herter's work, which is of especial value when the clinical significance of indol absorption is to be considered, I will only state that I agree with his conclusion that indol does not ordinarily exert highly toxic effects even when absorbed in unusually large amounts.

My own experiments on indol and skatol relate merely to their effect on arterial pressure. The indol used in my experiments was made according to Nencki's synthesis, acting with dichlor-ether on anilin. I believe that pure indol is more easily secured in this way than from putrefying fibrin. I have found that when injected in doses of 0.1 gramme into the jugular vein of the dog it produces no effect on arterial pressure. In frogs, as pointed out by Christiani,* it produces convulsions similar to those caused by phenol. What has been said of indol holds also for skatol, which has been fed to a dog weighing 55 kilos at the rate of 30 grammes in 21 days without any serious effect.† In my experiments no change of arterial pressure was produced by jugular injections of 0.1 gramme. In fact, I am satisfied that 20 times as much of either of these substances as are excreted daily by a man of 70 kilos weight may be injected at one time into the jugular vein of a dog of 4 kilos without producing an appreciable effect on the circulation or respiration. Indol, however, is much the more important of the two, as skatol, though formed in larger quantities, is absorbed only in traces.‡

When we consider, therefore, the amounts in which any of these substances could probably be formed under the most favorable circumstances, and compare these with the quantities which have been administered empirically or experimentally, we cannot but feel that to account for the symptoms in acute cases of intoxication something more active is necessary.

The third class of substances comprises putrescin, cadaverin and ethylidendiamin, all belonging to the diamins. Udránzsky and Baumann § have fed both putrescin and cadaverin to dogs in large doses without effect. Grawitz || has shown that they are both capable in 2.5 per cent solution of producing severe inflammation and necrosis,

* Loc. cit.

† Mester, *Zeitschr. f. physiol. Chem.*, xii, 130; Brieger, ibid., iv, 414.

‡ Brieger, ibid., iv, 241.

§ Ibid., xv, 77.

|| Virchow's *Archiv*, cx, 1.

while Behring * has found cadaverin, taken in large doses, poisonous to mice, guinea-pigs and rabbits. The substance found by Kulneff † in a case of gastroptosis is probably ethylidendiamin. It is more poisonous to mice and guinea-pigs than to frogs. In the former it causes lachrymation and salivation followed by violent dyspnœa, lasting until death, which follows in 24 hours or more. So of these substances it may be said that the first two are not extremely toxic and the chemical position of the last is still uncertain.

Of the various toxins which are known to be formed by the action of bacteria, we have not definite knowledge enough to speak until their principles are more completely isolated so that they can be studied as individuals. Of these, however, many are albumoses or of proteid nature and are destroyed according to Nencki by various digestive juices.‡

As the first three classes of these substances differ from what we commonly have in mind when we speak of poisons, so do the symptoms which they are supposed to produce in the so-called autointoxications differ from the toxic picture we see in a case of ileus or acute intestinal obstruction.

We know that the chief symptoms of ileus, such as pain, vomiting, cold clammy sweat, pallid and shrunken features, with possibly subnormal temperature and ultimate complete muscular relaxation, all of which often result in death within one or two days, can be simulated by poisons formed by bacterial activity, and that, too, within a comparatively few hours as, for instance, by the tyrotoxicon of Vaughan.§ Lépine and Molière ‖ have occasionally observed in cases of intestinal occlusion symptoms like those seen in atropin poisoning, namely, dilated pupils and marked redness of the skin, and these authors surmise that death in these instances may be in some degree due to autointoxication from absorption of ptomaines from the intestine.

* *Deutsche med. Wochenschr.*, 1888, No. 24.

† *Berl. klin. Wochenschr.*, 1891, p. 1071.

‡ Ransom (*Deutsche med. Wochenschr.*, 1898, p. 117), however, finds that tetanus toxin passes in large part unchanged through the alimentary canal, its harmlessness when administered by the stomach being due to incapacity of the stomach and intestine to absorb it. Behring (ibid., p. 662) considers that other proteid-like bacterial toxins behave in the same way.

§ *Zeitschr. f. physiol. Chem.*, x, 146.

‖ Cited from Eichhorst, Darmstenose, *Real-Encyclop. d. gesamm. Heilk.*, iii edit., v, 430.

It is not my purpose to offer a chemical theory in explanation of any of these various symptoms that arise in the course of an acute and complete obstruction of the intestinal canal at different points in its course. It is my object rather to present a chemical study of the intestinal contents in cases of complete obstruction of the small intestine in order to learn whether other or more powerful poisons than the putrefactive products already isolated can be found under such circumstances. Such poisons if present must exert their action and play their part, be it great or small, in the symptomatology of ileus; certainly the substances so far observed in the intestinal canal are not sufficiently toxic to account for any of the symptoms observed in intestinal obstruction. On the other hand, "shock" and similar expressions are far from giving a rational explanation of the condition described.

When we consider the chemical and physical conditions which exist in a case of this kind we find, first, a closure of the bowel, it may be by hernia, volvulus, intussusception or pressure, but the effect is to convert so much of the digestive tract as may be above the constricted portion into a closed thermostatic tube containing culture materials in the shape of proteids, carbohydrates, etc., kept at body temperature and infected by a varied bacterial flora, air being excluded. In this respect, the conditions are similar to an experiment conducted in the laboratory, where the same materials are used and inoculated with intestinal bacteria, but with this striking difference, that in the former case the tube is composed of animal membrane through which many of the products may pass by absorption, to be taken up later by the portal system and if unchanged in their passage through the intestinal wall (as pointed out before in the case of peptone), perhaps to be oxidized or otherwise changed by the liver cells before reaching the tissues. So that for a chemical theory not only would poisons have to be formed, but in order to produce alarming effects they must be of such a composition that they are not destroyed by the liver, or they must be produced in such quantities that the liver is unable to destroy them as fast as they are absorbed.

For the purpose of this research lecithin was chosen, a substance which is a constituent of all food materials and is widely distributed

in nature. The products formed by its decomposition are not only in some instances of extreme toxicity, but also capable of positive detection and identification. It has been found as a constant accompaniment of cell life, animal and vegetable, but chiefly in brain and nerve tissue, yolk of eggs and the germinating sprouts of plants, to a lesser degree in milk, muscles, etc.

Chemistry and Fate of Lecithin in the Economy.—It has been known for a long time that there are different lecithins according to the fatty-acid radicle contained, but more recently Lippman * found two lecithins in beet residue, one of which gave cholin on decomposition and the other betain; he has therefore suggested that we may have different lecithins depending on the interchangeability of the basic radicle, as we have different lecithins according to the acid radicles present.

We know that these complex molecules split up into different compounds with different arrangement of their component radicles according to the agents employed, but as a result of chemical action and putrefactive processes it has been abundantly shown that lecithin breaks up into glycero-phosphoric acid, fatty acids and basic bodies.

As regards the decomposition and fate in the economy of the different radicles composing the lecithin molecule, Bókai considers it analogous to the fats, and states that lecithin is decomposed during the digestive processes into glycero-phosphoric acid, fatty acids and cholin, and that these products are severally absorbed. According to this view it might be dangerous to consume a great deal of food rich in lecithin (eggs for instance) as cholin is certainly not a harmless substance. Bókai † subjected lecithin to the action of the pancreatic ferments and found that it was split up as above, but he mentions also that bacterial agency was not excluded. From the more recent experiments of P. v. Walther,‡ it seems fair to assume that some lecithin may be absorbed without decomposition, as he always found it present in the chyle of the dog to the extent of from 0.03-0.096 per cent. Hasebroek § has shown in putrefactive experiments, practically anaërobic as he used slime from the river Ill as the source of bacteria, that under these conditions cholin is broken up into methylamin, carbon dioxide and methane.

* *Ber. d. deutsch. chem. Gesellsch.*, xx, 3206. See also E. C. Shorey, *Journ. Amer. Chem. Soc.*, xx, 113.

† *Zeitschr. f. physiol. Chem.*, i, 162.

‡ *Arch. f. Anat. u. Physiol.*, physiol. Abth., 1890, p. 329.

§ Hasebroek, *Zeitschr. f. physiol. Chem.*, xii, 148.

In the case of the lecithin under consideration, which is much the more common form in foods, the basic body contained in its molecule is almost wholly cholin * or trimethyl-oxyethyl-ammonium hydroxide. The composition of this base is

$$N \begin{cases} (CH_3)_3 \\ CH_2 - CH_2.OH \\ OH. \end{cases}$$

It is readily oxidized to a highly poisonous compound isomeric with muscarin and, on losing a molecule of water, a process which may easily occur in the intestine, it yields the almost equally poisonous compound neurin. Neurin is trimethylvinyl-ammonium hydroxide and has the composition,

$$N \begin{cases} (CH_3)_3 \\ CH = CH_2 \\ OH. \end{cases}$$

The intimate relation between cholin and neurin is further shown by the fact that, as proved by E. Schmidt, neurin can be changed back to the oxyethyl compound, cholin. It has further been shown by Schmidt † that cholin chloride is decomposed almost entirely by putrefactive action, at 20-30° C., at the end of 14 days yielding large quantities of trimethylamin and a small quantity of a base whose platino-chloride is similar in crystallization and solubility to the neurin salt, and also agrees with neurin in its physiological action. When decomposition was carried on for ten days at 30-33° C. neither cholin nor neurin were present, nor could the presence of trimethylamin be determined with certainty. There can be but little doubt that the crystals isolated by Schmidt from solutions of neutral cholin chloride, which had been infected with hay infusions, consisted in reality of the chloride of neurin, and we must therefore regard the conversion of the relatively non-toxic cholin into the highly poisonous neurin as being within the power of perhaps numerous varieties of bacteria. Hasebroek, as mentioned in connection with lecithin, found on treating cholin solutions with sewage from the Ill, that it was entirely decomposed, yielding methane and carbon dioxide. The solution on treatment with alkali gave an odor of methylamin, and Brieger found that a cholin solution, after the action on it of putrefactive bacteria, gave trimethylamin on treatment with alkali. It will be seen that strikingly different results have occurred from putre-

* Strecker, *Anal. d. Chem. u. Pharm.*, cxxiii, 353.
† *Archiv d. Pharmacie*, ccxxix, 481.

factive experiments on cholin. This, however, is to be expected as the flora in many cases is entirely different, but all observers agree that in the examination of the products of putrefaction whenever cholin is present neurin is also present, although it may be in traces only.

In reference to the toxicity of these substances it has been shown that cholin, previously considered non-toxic, is fairly active, since Gaehtgens * has proved that 0.59 gramme produced almost instantaneous death in a cat. It has been further shown that cholin chloride produces the same muscarin-like symptoms as neurin although the latter are much more intense. Brieger † found that 0.005 gramme of neurin chloride would produce the same symptoms in a rabbit as 0.1 gramme of cholin chloride. He further found that the fatal dose of cholin per kilogramme of rabbit was 0.5 gramme or ten times that of neurin. Boehm ‡ considers the curara-like, paralyzing action of cholin to be like that of artificial muscarin, but the latter is 500 times more toxic.

If therefore we take it for granted that putrefaction takes place in the intestinal canal during obstruction, the toxicity of the substances formed will depend upon the material present and the character of the intestinal flora. It may be that at the time of obstruction the canal is comparatively free from those bacteria which would give rise to toxic substances, or, on the other hand, it may be highly infected.

If the lecithin contained in the food is decomposed in such a way as to give rise to cholin and possibly to neurin we may demonstrate their presence. On the other hand, failure to demonstrate the presence of cholin would not prove positively that the decomposition does not go on in this way; since, as in all experiments on the digestive tract, the substances formed are either further modified or are absorbed so rapidly that it is almost impossible at any one time to obtain a sufficient quantity for positive identification. This difficulty is naturally greatly increased when two or three days are allowed to elapse between feeding and the removal of the intestinal content. The intestinal content is then very small and contains so much bile that it is very difficult to handle. Out of six experiments on dogs in

* *Dorpater med. Zeitschr.*, 1870, i, cited from Boehm, *Arch. f. exp. Pathol. u. Pharmakol.*, xix, 87.

† Ueber Ptomaine, i, 39.

‡ *Arch. f. exp. Pathol. u. Pharmakol.*, xix, 87.

only one was I able to obtain a sufficient amount of a platinum salt for analysis.

It was my intention, in these experiments, to determine whether the lecithin content of the food could give rise to cholin and possibly neurin by decomposition in the intestine in cases of obstruction. The dogs used were therefore fed for two or three days before the operation of closing the intestine was performed on yolk of eggs, which is very rich in lecithin.

The following protocol from my notebook will serve to illustrate the entire series of four experiments:

Expt. 3. Friday, March 29, 2 P. M., anæsthetized dog, male, weight 55 lbs. Placed ligature around intestine just above ileo-cæcal valve. Animal had been fed for three days previously on yolk of eggs. Saturday, March 30, 6 P. M. Animal quiet, does not seem very sick, drinks well but does not eat. Urine of sp. gr. 103.2, acid in reaction, no albumin, strong indican reaction. Sunday, March 31. Dog drinks but does not eat, appears much the same, urine 274 cc., sp. gr. 102.8, reaction acid, no albumin. Indican reaction strong. Monday, April 1. Dog seemed better, but about 2 P. M. managed to tear open the incision in the abdominal wall, and in consequence a loop of the intestine escaped. Dog was killed with chloroform and an autopsy made. Urine for this day up to this time, 160 cc., sp. gr. 103.1, reaction acid, strong indican reaction. It may be said that in no case was there any marked anuria, as the dogs drank freely and did not vomit. As far as the indican reaction was concerned it was strong, but not much more so than I have seen in apparently healthy dogs.

It must be remembered that the indican reactions as usually made cannot be considered quantitative, as the color is produced by oxidation of the indoxyl which cannot be regulated to give quantitative results, as the same agent at the same time produces indigo red and indigo white. I consider Baumann's the best test, namely, equal volumes of urine and strong hydrochloric acid with a few drops of ferric chloride, as there is less chance of over-oxidation by this method.

Autopsy.—Evidences of peritonitis; some excess of peritoneal fluid containing flakes of fibrin, intense venous congestion. This was found to be due to perforation at point of ligature. There were slight adhesions between neighboring intestinal loops. Renal cortex much engorged, papillæ pale, capsule non-adherent. Liver hyperæmic, consistence nor-

mal, gall bladder distended, contents green; adjacent tissues stained yellow. Spleen hyperæmic, veins on surface distended. Heart, veins on surface distended, otherwise normal. Lungs, highly pigmented; some calcareous nodules; otherwise normal. Stomach contents small, reaction acid, whole internal surface hyperæmic, pyloric third stained yellow. Two small ulcerations in cardiac portion about middle of inferior curvature.

Intestinal content small, reaction acid, intensely bile-stained and whole surface hyperæmic. The acid character of the contents continued to within 15 inches of ligature and this lower portion of the ileum was very dark and had apparently lost all tone. It was filled with fæcal material, bright green in color. The most of the mucous surface of the ileum was highly congested and in the lower portion it was easily separable.

In all the experimental cases, except the foregoing, in which there was perforation at point of ligature, the tendency, with ordinary aseptic precautions, is toward recovery. Plastic processes connect the intestinal walls around ligature, necrosis occurs at the point of ligature and a passage is usually established in 5-7 days. This was a source of disappointment in the earlier experiments as, in waiting for the full effects of obstruction in order to obtain as much material as possible, the experiments failed because of the escape of material through newly formed passages, re-establishing the continuity of the intestine. In those animals which were chloroformed in from 70-80 hours after ligature, there was no abnormal appearance of the internal organs, with the exception of the kidneys, in which there was much engorgement of the capsular veins and intense hyperæmia of the cortex, though the papillæ remained pale.

Microscopic Examination.—Kidneys showed infiltration of Bowman's capsule, cloudy swelling of epithelium of convoluted tubules, some necrosis of the epithelium and tube casts.

Chemical Treatment of the Intestinal Contents.—As it was my intention to ascertain by the presence or absence of cholin, whether there had been decomposition of lecithin during the obstruction of the intestine, it was first necessary to choose a method that would totally obviate, if possible, the chemical decomposition of the lecithin in the analytical processes employed. The most suggestive work in this connection is that of Marino Zuco,* who claims that, by the methods of the toxicologists, it is possible to obtain cholin from fresh tissues, blood, etc., and that the

* The so-called Ptomaines in Relation to Toxicological Researches. Abstr. in *Journ. Chem. Soc.*, xlvi, 342.

cholin thus found originates from the splitting up of the lecithins under the influence of the acids and alkalis.

The intestinal contents of the animal described in Experiment 3 were removed with the aid of as little water as possible. Together with the water added the intestinal contents amounted to 280 cc. and had an acid reaction. The whole was treated with four times its volume of absolute alcohol and left with occasional shakings for 18 hours. It was then filtered and being still acid was evaporated in a large dish on the water-bath, the temperature of the fluid not going above 70° C. at any time; absolute alcohol was occasionally added to carry off the balance of water at the same low temperature. When the material had been reduced to a thin syrup it was mixed with a large quantity of powdered glass, evaporated to dryness in vacuo at 45°-50° C. and then placed in a Soxhlet extracter and thoroughly extracted with ether. This removes all of the lecithin, cholesterin and fats, a great deal of coloring matter, extractives, etc. It is of course understood that the method of treatment was governed by the substance sought. If no cholin was present my question could not be answered in the affirmative; on the other hand, the varying statements in reference to the ease or difficulty with which lecithin is decomposed made it imperative that the possibility of its decomposition should be avoided. Marino Zuco is the chief authority for the statement that lecithin is easily decomposed by analytical methods, and the method devised by him includes digesting on the water-bath for 24 hours at 70°. It is apparent therefore that much less injury must result from evaporating the fluid at the same temperature in one-eighth of the time. Further, I find that drying at first and extracting with ether in Soxhlet's apparatus much facilitates succeeding operations.

Schulze and Steiger * claim that in the examinations of certain seed contents made by previous investigators, all the lecithin was not extracted by ether, and they make these deductions from the fact that after shaking the finely ground seeds in a flask with a quantity of ether, allowing it to stand for some hours and then repeating the process two or three times, they were still able to obtain lecithin. This, however, is quite different from 36 hours' extraction in Soxhlet's apparatus, as in my experiment. After extracting with ether for this length of time one may rest assured that every trace of lecithin has been removed.

After the substance had been extracted with ether as described, it was removed, dried, and extracted with absolute alcohol, acidified with hydrochloric acid. Of the more common putrefactive bases only the chlorides

* *Zeitschr. f. physiol. Chem.*, xiii, 365.

of cholin and neurin are soluble in absolute alcohol and also the chlorides of some of the amines. The alcoholic extracts were united and evaporated to a small bulk and were then treated with an alcoholic solution of platinum chloride, the precipitate was thoroughly washed on a filter with alcohol and ether, and was then dissolved off with cold water, in which it proved to be almost entirely soluble. This solution of the platinum chloride double salt was then decomposed with hydrogen sulphide, was boiled and filtered, and a portion of the filtrate was neutralized and tested with the following alkaloidal reagents:

Reagents.	Reaction.
Phosphomolybdic acid	abundant yellow caseous ppt.
Phosphotungstic acid	white ppt. crystalline.
Potassium bismuth iodide	dark brown pulverulent ppt. somewhat sol. in excess.
Potassium cadmium iodide	white ppt. sol. in excess.
Potassium mercuric iodide	yellow crystalline ppt.
Potassium iodide and iodine	dark brown ppt.
Bromine water	reddish ppt.
Mercuric chloride	ppt. white, gradually becoming crystalline.
Gold chloride	yellow granular ppt. sol. on heating.
Platinum chloride	slight cloudiness.
Tannic acid	white finely flocculent ppt.
Picric acid	no precipitate.

The balance of the filtrate was now evaporated down and precipitated with gold chloride and filtered, the gold salt decomposed with H_2S, and the solution boiled and filtered. The filtrate gave the following alkaloidal tests:

Reagents.	Reaction.
Phosphomolybdic acid	abundant yellow ppt.
Phosphotungstic acid	white crystalline ppt.
Potassium bismuth iodide	dark brown pulverulent ppt. somewhat sol. in excess.
Potassium cadmium iodide	slight ppt. sol. in excess.
Potassium mercuric iodide	yellow crystalline ppt.
Potassium iodide and iodine	dark brown ppt.
Bromine water	reddish ppt.
Mercuric chloride	white ppt. gradually becoming crystalline.
Gold chloride	yellow granular ppt.

On the basis of the above reactions the solution was considered to contain only pure cholin chloride, and it was therefore evaporated down, taken up in absolute alcohol and precipitated with alcoholic platinum chloride. The yellow precipitate of double salts was filtered off, washed with alcohol and ether, dried in vacuo at 100° and analyzed.

0.1457 gramme of this salt gave 0.0463 gramme platinum or 31.77 per cent. For cholin: Theory requires 31.64 per cent platinum. Found 31.77 per cent. platinum.

The presence of cholin in the intestinal contents of the animal experimented upon is therefore proven.

On the Presence of Neurin in the Intestinal Contents Examined.—From the fact that there was a precipitate with potassium cadmium iodide, tannic acid and also a slight precipitate with platinum chloride, I considered there was neurin as well as cholin present in my final solutions. Gulewitsch,* in one of the most complete chemical studies of cholin which has yet been published, draws attention particularly to the fact that Brieger and others, working with such solutions and using tannic acid to distinguish qualitatively between cholin and neurin, fell into an error in using this reagent. Cholin chloride, in acid solution, will not give a precipitate with tannic acid, but in neutral solution invariably does so. I have stated previously that neurin has been considered invariably to accompany cholin. It is possible that, using only qualitative tests, an error may have occurred when experimenters did not note whether the solution of chlorides was neutral or acid.

It has already been stated that when the platinum salt of cholin was dissolved on the filter by the free use of cold water a small quantity of a platinum double salt remained undissolved. This salt was, however, found to dissolve in hot water and when the solution had cooled, small, yellow octahedral crystals were deposited which resembled crystals of the corresponding salt of neurin as described by Gulewitch † in his recent paper on neurin and its compounds. Now, these octahedra could not consist of the platinum salt of one of the amines or diamines, for, the former were excluded by testing the original solution from which the cholin and neurin were precipitated by the chlorides of platinum and gold, and the latter were excluded by the fact that their platinum double salts differ in crystalline character from the octahedral crystals here described. On warming gently the original solution with a slight excess of alkali or of a solution of sodium bicarbonate it was not possible to detect the odor of an amine. Unfortunately, this platinum salt, soluble only in hot water, was not obtained in sufficient amount to warrant decomposing it and performing pharmacological tests with it. Nevertheless, I consider the presence of neurin in the intestinal contents, under the experimental conditions set forth in this paper, as almost a certainty.

† *Zeitschr. f. physiol. Chem.*, xxvi, 175.

* *Zeitschr. f. physiol. Chem.*, xxiv, 513.

The following is a tabular statement of the reactions for cholin obtained with the intestinal contents of the dogs used in Experiments 1, 2 and 4, after these contents had been subjected to the chemical treatment already described. In none of these experiments was enough of a salt of cholin obtained to warrant an analysis. The reactions are stated very briefly, but coincide entirely in appearance and character with those given under Exp. 3, and prove that cholin was present in the intestines of all of these animals though in less amount than in those of the animal used in Exp. 3:

Reagent.	Exp. 1.	Exp. 2.	Exp. 4.
Phosphomolybdic acid......	ppt.	ppt.	ppt.
Phosphotungstic acid	ppt.	ppt.	ppt.
Pot. bismuth iodide	ppt. brown.	ppt. brown.	ppt. brown.
Pot. cadmium iodide.......	ppt. white.	ppt. white.	ppt. white.
Pot. mercuric iodide........	ppt. yellow.	ppt. yellow.	ppt. yellow.
Pot. iodide and iodine.......	ppt. brown.	ppt. brown.	ppt. brown.
Bromine...................	ppt. brown.	ppt. brown.	ppt. brown.
Mercuric chloride...... ...	ppt.	ppt.	ppt.
Gold chloride	ppt. yellow.	ppt. yellow.	ppt. yellow.
Platinum chloride..........		slight cloudiness.	slight cloudiness.
Tannic acid................			ppt.
Picric Acid.................			

The absence of the precipitates with tannic acid in Exps. 1 and 2 were due most probably to the fact that the solution was acid, whereas in 3 and 4 it was neutralized before tests were made.

On the Occurrence of a Ptomaine accompanying the Cholin and Neurin.—In Experiment 1, in which the dog died during the night and was examined the following morning, a ptomaine was found which possessed the following characteristics. Its hydrochloride is very soluble in alcohol and water and crystallizes in fine needles. It gave all the reactions of cholin, but both the platinum and gold salts were quite insoluble in cold water and difficultly soluble in hot water, the gold salt being more easily soluble than the platinum one. The platinum salt, which had been dissolved in hot water and filtered clear into a watch glass, on cooling gave a deposit, which, under the microscope, had the appearance of small, bright yellow spheres which reacted towards light like crystals. An iridescent scum formed on the surface of the water from gradual decomposition of the salt. The gold salt was dissolved in hot water and acidulated with hydrochloric acid, and, as it appeared to be reducing, the liquid solution was quickly filtered and cooled. On cooling it showed a fine yellow granular deposit of spheroidal crystals. On examination the following morning the deposit was quite

dark and a beautiful mirror had been formed on the sides and bottom of the crystallizing dish as well as on the surface of the liquid. Both the platinum and gold salt, therefore, are easily reduced compounds. It is also to be noted that the free base has a penetrating, sweetish odor, and that it is easily oxidized to a brown resin when its solutions are left exposed to the air.

The amount of this ptomaine at my disposal was insufficient for establishing its identity. It agrees in some of its properties, though not in others, with a ptomaine, $C_8H_{13}N$, obtained by Gautier and Étard * from putrefying mackerel and from the decomposing flesh of the ox and horse. It resembles, perhaps, more closely a base, $C_{10}H_{15}N$, which has been isolated from sea-polyps in an advanced stage of putrefaction by de Coninck.† The hydrochloride of de Coninck's base forms fine, deliquescent needles, changes to a brown resin when exposed to the air, and both the platino- and auro-chloride are decomposed by boiling water.

In the intestinal contents of animal No. 4 apparently the same base was present, for, on shaking out the ether from the Soxhlet apparatus with acidulated water a few milligrammes of a gold salt were obtained which resembled the gold salt already described, and on heating, burnt with a smoky, oily flame which gave off a disagreeable odor.

SUMMARY.

My experiments lead me to believe that complete occlusion of the small intestine at its lower end will give rise to the occurrence of cholin, neurin and perhaps other bases, provided the food taken contains any considerable quantity of lecithin. It is not improbable that still other poisons are formed by bacterial action from other constituents of the food in cases of intestinal obstruction. While cholin would have to be absorbed in relatively large amounts to exert a marked toxic action in human beings it is otherwise with neurin, which is many times more intense in its action and must be classed with the exceedingly active poisons. It has been shown both by the experiments of Schmidt and Weiss and also by those recorded in this paper that the poisonous neurin may be formed from cholin by bacteria. In its physiological action neurin agrees closely with muscarin; especially to be noted here is the paralytic action on the heart and its power to

* Vaughan and Novy, Ptomaïns and Leucomaïns, 3rd edit., 316.
† Ibid., 318.

increase the intestinal movements to such an extent that continual evacuations occur. Whether the ptomaine which was found by me is poisonous I cannot yet say. It must be considered proved, however, that highly toxic substances may arise in the intestinal canal during its complete occlusion. The method of treating cases of intestinal obstruction, before surgical means are resorted to, namely, washing out the stomach and as much of the gut as possible often reduces the violent paristalsis and this is due, perhaps, to the removal of substances out of which irritating and toxic products are formed by bacteria.

In conclusion, I would remark that our knowledge of the fate of lecithin in the digestive canal under normal conditions is very deficient. The assumption that it is saponified by the fat-splitting enzyme of the pancreatic juice, thus yielding cholin, glycero-phosphoric acid and fatty acids, rests on the work of Bókai * in 1877 and, as that investigator himself admits, without excluding bacterial action. This omission throws grave doubts on the results. If the assumption of Bókai be correct, caution must be observed in the use of some foods that have been considered most nutritious and healthful; for instance, the ingestion of a meal made up largely of eggs would hardly be without danger because of the poisonous action of the large quantity of cholin liberated from the lecithin and the probability of the formation of the highly poisonous neurin.

It is my purpose in the near future to examine this question with the help of modern methods.

* *Zeitschr. f. physiol. Chem.*, i, 157.

ON THE VALUE OF UROTROPIN AS A URINARY ANTISEPTIC WITH ESPECIAL REFERENCE TO ITS USE IN TYPHOID FEVER.*

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In a previous number of this Journal † the writer published an article "Upon the Presence of the Typhoid Bacillus in the Urine," and, as the result of observations upon 38 cases of typhoid fever, drew the following conclusions: (1) Typhoid bacilli were demonstrated in 9 out of the 38 cases (about 25 per cent). (2) The bacilli, when demonstrated, were always present in large numbers, and in practically pure culture. (3) The bacilli appeared first in the later stages of the disease, and persisted, in the great majority of cases, far into convalescence. The urine of typhoid patients should, therefore, not only be rigorously disinfected during the disease, but should also be carefully supervised during convalescence. (4) The typhoid bacilli were practically always associated with albuminuria and the presence of renal casts. On the other hand, urines containing considerable amounts of albumin and casts in large numbers often showed no typhoid bacilli. (5) Irrigation of the bladder with antiseptic solutions offers a possible means for removing permanently the bacilli from the urine.

Since the publication of that article two further contributions to the subject have come to my notice, the first by Horton-Smith and the second by Petruschky. The work of Horton-Smith appeared in 1897 in the *Transactions of the Medical and Surgical Society* of London, and should properly have been included in the literature mentioned in my previous article. Horton-Smith examined the urines

* Final report of work done under the provisions of the Dalton Scholarship for 1897-8.

† *Journal of Experimental Medicine*, 1898, iii, 349.

of 7 typhoid patients, with 3 positive results. His conclusions were in general very similar to mine. Horton-Smith also pointed out the fact that the typhoid bacilli can be demonstrated oftentimes in cover-glass preparations from the urine; and, further, that the organisms may be so numerous as to render the urine distinctly turbid.

Petruschky * examined the urines of 50 typhoid patients with but three positive results. Petruschky followed the cases into convalescence, and found that the first case retained the bacilli two months after the temperature had reached the normal point; that in the second case the bacilli were still present a month and a half after the disappearance of fever; that in the third case the organisms had disappeared eight days after the beginning of convalescence. The fact that the organisms were present in enormous numbers was strongly emphasized by Petruschky. According to his calculation a single cubic centimetre of urine, in one case, contained 170,000,000 typhoid bacilli.

Finally, through the courtesy of Dr. Harvey Cushing, I am permitted to report briefly a most remarkable case observed recently at the Johns Hopkins Hospital. The patient, a man, had been in the Johns Hopkins Hospital five years before with an attack of typhoid fever. Ever since his discharge from the hospital he had had trouble with his urine. Investigation showed him to be suffering from a cystitis, and the typhoid bacillus was obtained in pure culture from his urine.

We see, therefore, in the light of the accumulated evidence, that our supervision of patients sick with typhoid fever has hitherto been very inadequate; that we must disinfect carefully the urines as well as the stools of typhoid patients; that the necessity for such disinfection and supervision does not cease with the fever, but must be kept up, oftentimes for weeks and sometimes for years.

In the previous article already referred to, the question of treatment for this condition was barely touched upon. In a single case the bladder was irrigated with antiseptic solutions, and the results demonstrated the comparative uselessness, as a remedy, of boric acid, and the effectiveness of corrosive sublimate. Irrigation, however, with its necessary catheterization, is not a method of treatment easily

* *Centralbl. f. Bakt.*, 1898, xxiii, 577.

applicable, and it was next determined to try the effect of the so-called urinary antiseptics, salol, benzoate of ammonia, urotropin, etc., given internally.

The number of cases of typhoid fever comprised in the present series is 66, and the number of specimens of urine examined 190. Of the 66 cases, 14 showed the presence of typhoid bacilli in the urine. The percentage of positive results is therefore somewhat smaller than in the previous series, a difference due possibly to the fact that, in the latter series, the urines were first examined at or about the beginning of convalescence; whereas, in the former, investigation was begun early in the disease. For this reason it is entirely possible that in a number of cases the urines had contained bacilli, but that these had disappeared spontaneously before investigation was begun. Every case showed at some time during the disease a positive typhoid serum reaction (Widal).

The methods employed in obtaining the urines, and the tests applied in the identification of the typhoid cultures, were the same as in the previous series.*

The positive cases, their course and treatment, may be outlined briefly as follows:

Case VIII. Clinical course severe. Relapse. Urine was examined on the 39th, 46th, 50th, 53rd, 57th, and 61st days. Salol was given, 10 grs. t. i. d. from the 42nd to the 55th day. The bacilli persisted, however, in large numbers, and the patient left the hospital with the condition unrelieved.

Case IX. Clinical course very mild. Bacilli present in the urine of 22nd and 28th days. The number of organisms was always small. Treatment: salol 10 grs. t. i. d. from the 26th to the 35th day. Urine of the 32nd and 35th days showed no bacilli.

Case X. Clinical course very severe. Relapse with streptococcus infection. Death. Bacilli present in urine in very large numbers on the 19th, 25th, 43rd, 76th, and 79th days. No treatment was instituted in this case.

Case XVI. Clinical course very severe, followed by cholecystitis. Cholecystotomy was performed and many stones of considerable size evacuated. Fluid from the gall bladder showed typhoid bacilli in pure

* Loc. cit.

culture. In this case salol was given in 10 grain doses three times a day from the 28th to the 35th day. The urine became dark on standing. In spite of the treatment, however, typhoid bacilli were found in large numbers in the urines of the 28th, 35th, 39th, and 43rd days. At the suggestion of Dr. H. F. Vickery it was then resolved to try urotropin, and the drug was given in doses of 10 grains three times daily from the 45th to the 54th day. Further examination of the urine was made on the 47th, 49th, 52nd, 57th, and 71st days, and not a typhoid bacillus could be demonstrated. Moreover, it is to be noted that upon the day of the last examination (71st day) 17 days had elapsed since the last dose of urotropin. The removal of the bacilli was, therefore, permanent, and not confined merely to the time during which the drug was administered.

Case XV. Clinical course moderately severe. Typhoid bacilli were found in large numbers on the 32nd day. Urotropin, 10 grains t. i. d., was given from the 33rd to the 38th day, and from the 45th to the 53rd day. The urines of the 35th, 38th, 45th, 53rd, and 67th days showed no typhoid bacilli. After 50 grains of urotropin the bacilli had disappeared, and two weeks after the administration of the drug had been stopped no bacilli had reappeared.

Case XXV. Clinical course very severe. Death. Typhoid bacilli found in urine of the 15th day. Case not treated.

Case XXVIII. Clinical course moderately severe. Typhoid bacilli in very large numbers found in urines of 34th and 38th days. Urotropin, 10 grains t. i. d., was given from the 38th to the 42nd day. On the 40th day no bacilli could be found—a fact which is the more remarkable when it is noted that the patient vomited four of the five doses given. The amount of urotropin actually absorbed must have been very small, still its action was effective. On the 47th day—5 days after urotropin was stopped—no bacilli had reappeared in the urine, and a similar result was obtained upon the 108th day.

Case XXXIII. Clinical course moderately severe. Typhoid bacilli present in large numbers on the 25th day. Urotropin, 10 grains t. i. d., was given from the 26th to the 27th day. On the 27th day, after only 20 grains had been given, the bacilli had disappeared. Further examination on the 70th day showed no bacilli.

Case XXXIV. Clinical course moderately severe. Bacilli present in urines of the 12th and 13th days in large numbers. Urotropin, 10 grains t. i. d., from the 13th to the 16th day. On the 14th day, after 30 grains of the drug, the bacilli had not entirely disappeared, but the number

had been reduced from millions to hundreds per cubic centimetre. On the 15th day, after 60 grains of urotropin, no bacilli were seen. On the 20th day there was also a negative result. On the 38th day, however, I was much surprised to find that, not only had the bacilli returned to the urine, but there was also a mild cystitis—a condition which had not been present previously. Urotropin was at once resumed, and given from the 38th to the 47th day. On the 40th day no bacilli could be cultivated from the urine, and the evidence of cystitis had largely disappeared. The urines of the 42nd and the 47th day showed no bacilli. On the 50th day, three days after stopping urotropin, the bacilli had again reappeared, though in comparatively small numbers. Urotropin was again begun, this time in 20 grain doses three times daily. After 40 grains no bacilli were found. On the 55th day the drug was again stopped. On the 64th day the bacilli had not reappeared.

Case XLIII. Clinical course severe with relapse. Typhoid bacilli present in moderately large numbers on the 14th, 23rd, and 37th days. Urotropin, 10 grains t. i. d., was given from the 38th to the 40th day. Urine of the 40th day, after 40 grains of the drug, showed no bacilli.

Case XLIV. Clinical course moderately severe. Typhoid bacilli first found on 38th day. Urotropin, 10 grains t. i. d., from the 39th to the 43rd day. Urine examined on 43rd, 50th, and 56th day, but no bacilli were found after 40 grains of urotropin had been taken.

Case LIII. Clinical course very severe. Typhoid bacilli in moderately large numbers on the 61st and 62nd day. On the 64th day urotropin, 10 grains t. i. d., was begun and continued till the 73rd day. On the 65th day (after 40 grains) the bacilli still persisted in small numbers (hundreds to the cubic centimetre). On the 66th day (after 90 grains) no bacilli could be found. On the 68th day (after 150 grains) a cubic centimetre of urine showed two colonies of typhoid bacilli. On the 70th day (after 210 grains) no bacilli were found. Examinations made upon the 78th, 79th, and 92nd days were similarly negative.

Case LV. Clinical course moderately severe. Urine of 21st and 22nd days showed typhoid bacilli in enormous numbers, as well as a cystitis of moderate severity. Urotropin, 10 grains t. i. d., was given from the 22nd to the 31st day. On the 24th and 27th days no bacilli could be demonstrated. Moreover, the evidences of cystitis had decreased markedly. On the 34th day a few bacilli were found, but they disappeared without treatment on the 35th day. The patient now left the hospital, and was instructed to continue the urotropin as a precautionary measure from the 36th to the 43rd day. Further examination on the 50th day showed no bacilli.

Case LVII. Clinical course extremely severe and complicated by a pneumonia. Death. Bacilli first found in urine obtained at autopsy.

To sum up briefly, we find that—

(1) Of 66 cases of typhoid fever, 14 showed the presence of typhoid bacilli in the urine.

(2) Eleven cases were submitted to treatment with the following results:

(*a*) Two cases received salol alone, and in one instance the bacilli disappeared.

(*b*) One case received first salol, with negative results. Urotropin was then substituted for the salol, and the bacilli disappeared almost immediately.

(*c*) Nine cases (including the case treated first with salol) were treated with urotropin, and in every instance the use of the drug was followed by the disappearance of the bacilli. Moreover, this remarkable result was accomplished in eight out of the nine cases by 60 grains or less of the remedy. A single case (LIII) required 200 grains to remove the organisms. Seven cases were followed for 7, 9, 13, 14, 17, 19, 43 and 66 days, respectively, after the administration of urotropin was stopped, and no bacilli had reappeared. It is fair to assume, therefore, that in these cases the removal of the typhoid organisms was permanent. One case was not followed subsequent to the omission of the urotropin.

Aside from the question of treatment, few conclusions different from those stated in the previous article can be drawn.

As already pointed out by Horton-Smith, the bacilli are often so numerous as to cause distinct turbidity in the urine. For this reason a turbid urine (freshly passed), especially if acid in reaction, should always be regarded with suspicion. Moreover, if such a urine when examined microscopically, either fresh or stained as recommended by Horton-Smith, shows, as it often does, the presence of bacilli, it can be prophesied with great probability that the organisms are typhoid bacilli, and the results of the cultures can be foretold with considerable certainty.

THE NATURE AND ACTION OF UROTROPIN.

Urotropin was first introduced in 1894 by Nicolaier,* and is said to be formed by the action of four molecules of ammonia upon six molecules of formaldehyde. It appears in the urine partly as urotropin and partly as formaldehyde. Urine containing urotropin gives with bromine water a yellow-brown precipitate of dibrom-urotropin. It appears in the urine as early as fifteen minutes after its administration, and can be demonstrated twelve hours after a single dose of 7½ grains. Indeed in rare instances the drug can be demonstrated for two weeks after its administration has been stopped.

Nicolaier claimed that urotropin would assist in the solution of uric acid concretions, and, further, would prevent the development of bacteria in the urine.

Casper † and Mendelsohn ‡ could not agree with Nicolaier as to the solvent powers of the drug, but as a urinary antiseptic they recommended it very highly, especially in suppurative pyelitis and cystitis. Casper also found urotropin valuable in so-called essential phosphaturia, and before all operations upon the urinary tract he used the drug to render the urine aseptic. Elliott § praises urotropin highly as a remedy in cystitis, and finds it much superior to salol, benzoate of ammonium, resorcin, napthalin, guaiacol, and boric acid. Elliott observed no ill effects whatever from the use of the drug, and this has been the experience of all other observers. Rarely as the result of large doses there may occur burning sensations at the neck of the bladder, with increased frequency of micturition, but these symptoms disappear immediately upon the omission of the drug or the reduction of its dose.

In my series of typhoid cases a large number had renal disturbance, as evidenced by the presence of albumin and casts in the urine, but in no case did the urotropin increase the gravity of the condition. In one case, as already stated, the drug caused nausea and vomiting, but this effect was due, I am sure, to the fact that, through a mistake,

* *Centralbl. f. d. med. Wiss.*, 1894, p. 897, and *Deutsche med. Woch.*, 1895, p. 541.

† *Deutsche med. Woch.*, 1897, Therap. Beilage, p. 75.

‡ *Berlin. klin. Woch.*, 1898, p. 48.

§ *North American Pract.*, 1897, ix, 451.

the remedy was given on an empty stomach, instead of in the proper manner, after food.

Cohn * reports great improvement in cases of chronic cystitis associated with enlarged prostate and urethral stricture. In cystitis following acute gonorrhœa, and in tubercular cystitis, there was no improvement.

Loebsch † used urotropin in the case of a woman who had large amounts of uric acid in the urine, and who, moreover, had symptoms of stone in the kidney. The use of urotropin was followed by complete recovery.

My own experience with urotropin in conditions other than typhoid fever is limited, but confirms in general the results obtained by others. In a later paper I shall hope to give a detailed account of these cases.

Returning to the question of the urine in typhoid fever, we draw, as the result of these investigations, the following conclusions:

(1) The urine of typhoid patients may contain typhoid bacilli in enormous numbers.

(2) The bacilli may persist in the urine for weeks, months, or even years, and thus constitute (*a*) a danger to the patient himself (cystitis, orchitis, and epididymitis?), and (*b*), what is much more important, a grave source of danger to the public health.

(3) The necessity for the rigid disinfection and supervision of typhoid urine is apparent.

(4) In 9 cases of typhoid fever in which the urine contained typhoid bacilli, urotropin never failed to remove the organisms. In 7 cases the elimination of the bacilli was permanent. In one case the omission of the urotropin was followed upon three separate occasions by the return of the typhoid organisms to the urine. The fourth attempt was successful. One case was not followed after the urotropin was stopped. There is therefore the possibility that in this case also the bacilli returned, but I do not regard this as probable.

(5) In the great majority of cases 60 grains of urotropin are sufficient to remove the typhoid bacilli. Exceptionally, however, as much as 200 to 300 grains are required.

* *Berlin. klin. Woch.*, 1897, p. 914.
† *Wien. klin. Woch.*, 1897, No. 12.

(6) Inasmuch as it is impossible, without bacteriological examination, to tell whether or not a urine contains typhoid bacilli, all typhoid patients should receive urotropin, 30 grains daily for 10 days, beginning with the third or fourth week of the disease, as convalescence approaches. Probably equally good results could be accomplished by the administration of small doses (10 grains daily) of the drug throughout the disease.

I take this opportunity of expressing my thanks to the members of the Visiting and House Staff of the Massachusetts General Hospital for the abundant clinical material placed at my disposal. I am also much indebted to the Director of the Pathological Laboratory, Dr. J. H. Wright, who has assisted me in every possible way in carrying out this work.

REGENERATION OF THE DORSAL ROOT FIBRES OF THE SECOND CERVICAL NERVE WITHIN THE SPINAL CORD.

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INTRODUCTION.

The object of the researches here described was to determine whether there is or is not, within the cord, regeneration of the fibres of the dorsal roots of the spinal nerves following the degeneration caused by injury to the roots between the spinal ganglion and the cord, and also to show the amount of this regeneration, if any should occur.

With this object in view experiments were conducted upon the second cervical nerve in the dog. The selection of this nerve was suggested by the following considerations: In the first place, the posterior root ganglion of the second cervical nerve in the dog lies normally outside the intervertebral foramen, so that experiments upon the roots are much easier and the risk of injuring the cord is much less than it would be were it necessary to open the vertebral canal. Moreover, the second cervical nerve in the dog is connected with the sympathetic system, not by a mixed ramus communicans, but by a gray ramus alone; a fact of some importance, as will be shown later.

Evidences of regeneration were sought for both by physiological and by histological methods. The present article deals with the physiological methods and the results obtained by them, while a discussion of the subject from a histological standpoint will appear later.

The literature of this subject is not extensive, and most of it dates from a period when the ideas concerning the relations of the nerve fibres and cells were less complete than at the present day. In the very brief summary of this literature that is here presented no especial attempt is made to criticise the older results in the light of newer

knowledge, since our object is simply to call attention to the work previously done in this field. On the clinical side, so far as the writers can ascertain, no satisfactory cases are reported for man of regeneration and return of function after lesions involving the destruction of any part of the central nervous system. On the experimental side, the subject of regeneration in the cord or brain has been studied more carefully by histological than by physiological methods. The efforts made to prove by physiological means the possibility of regeneration have been limited mainly to observations upon the return of sensation or voluntary movement. The results are therefore less certain and more open to double interpretation than would be the case if more exact experimental methods had been employed.

H. Müller (1) cut off the tails of lizards and tritons and demonstrated that anatomical and physiological regeneration of the spinal cord in the regenerating tail may take place, although incompletely.

Masius and Vanlair (2) also obtained regeneration and return of function in frogs after excising pieces of the spinal cord. In some cases the regeneration was complete.

Voit (3) claimed a regeneration and partial return of function after removal of the cerebral hemispheres of a pigeon. This claim has probably been disproved by later experimenters, and the fact that in Voit's case the animal never ate voluntarily nor showed signs of fear indicates that the return of function was not more complete than in the cases of Schrader, in which the entire cerebrum was undoubtedly lacking.

Brown-Séquard (4) described a return of function after section of the cord in pigeons and guinea-pigs. In the former the return was complete, in the latter only partial.

Dentan (5), Eichhorst and Naunyn (6) and subsequently Eichhorst (7) described voluntary movements in dogs after section of the spinal cord. But viewed in the light of the work of Schiefferdecker (8) and of the careful description of reflex movements furnished by Freusberg (9), it seems not impossible that these movements were of a reflex character.

In connection with his interesting microscopical work, Kahler (10) made a few experimental observations. He crushed the dorsal roots of the sixth and seventh lumbar and first and second sacral nerves in young dogs. The resulting anæsthesia and loss of reflexes persisted, except that, after a few months, a hard pinch of the toes appeared to cause pain. It is difficult to understand this latter result without supposing that the crushed nerves again formed connections in the spinal cord.

It thus appears that in the lower vertebrates a certain amount of return of function can follow a lesion in the central nervous system, while it is not yet decided certainly whether any such return is possible among higher animals.

METHOD OF INVESTIGATION.

In our experiments we made use of the second cervical nerve and a short description of the anatomical relations of this nerve are given first, as it may make clearer the subsequent account of the results of the experiments.

The dorsal and ventral roots of the second cervical nerve, after uniting in the intervertebral foramen, pass together to the ganglion, which lies about 2 mm. external to the foramen. Beyond the ganglion the nerve trunk divides into a dorsal and a ventral branch. The former in turn divides into three main branches. The ganglion and the origin of these three dorsal branches lie on the ventral surface of the m. rect. cap. (posticus) major, and, as they curve around the lower border of this muscle, these branches begin to diverge from one another. The main branch, n. occip. magnus, has the following cephalad course: Over the ventral surface of the m. complexus, through the substance of this muscle, emerging close to the median line of the neck: thence over the superficial surface of the m. temporalis to terminate in the m. levator auris longior and the integument of the region about the ear. A second branch passes beneath the larger branches of the third cervical nerve, but without communicating with this nerve, and enters the m. complexus, where its finer ramifications anastomose with similar branches of the third cervical nerve. The third branch, after passing beneath the m. complexus, enters the m. splenius, where its ramifications also anastomose with those of the third cervical nerve.

The ventral trunk passes between the m. longus capitis and the m. longissimus capitis, and then internal to the m. sternocleido-mastoideus. It gives off communicating branches to the n. accessorius and divides into the n. auric. magnus and smaller branches, which are distributed to the lower part of the neck.

The method employed in experimenting on this nerve was as follows: The continuity of the dorsal root fibres was destroyed by ligation of the roots between the ganglion and the cord. The animals were allowed to live for a variable period after the operation so that the

lesion might be followed by degeneration of all the nerve fibres entering the cord by the dorsal root, throughout their whole extent central to the point of ligation. After a period of not less than 88 days (the maximum being 151 days), the nerve was tested for return of function. Such a return would be indicated by the reflex variations in blood pressure, pulse rate and respiration, which might follow stimulation of the nerve in question. The nerve and cord were afterwards studied histologically.

As control experiments kymographic tracings were obtained, showing the variations due to stimulation of the second cervical nerve in two normal dogs. In one of these (Dog B) the roots were then ligated in the usual manner and the nerve again stimulated but with negative results. In all cases both the anterior and posterior roots were ligated, as it was impossible to separate them, a fact without significance in the experiment as only motor fibres occur in the anterior root.

Ligation of the roots.—The dog was etherized, the back of its neck shaved, well scrubbed and then washed with bichloride (1:1000). An incision was made about 8 cm. in length, extending from a point about 2 cm. above the protub. occipit. extern. down the mid-line of the neck. This incision passed through the skin and subcutaneous tissue, which were then retracted, exposing the n. occipit. magnus running to the ear. By separating and retracting the muscles and dividing the m. rect. capitis (posticus) major transversely, both the ganglion and the more central part of the nerve were exposed. After carefully freeing the roots from the surrounding tissue, a white silk ligature was passed around them and tied tightly. After a few moments the ligature was removed with a pair of fine, sharp scissors, leaving a constriction of the roots plainly visible at the point of ligation, the two parts of the nerve being united only by a tube of translucent connective tissue—the epineurium—while the continuity of the enclosed fibres was destroyed. The field of operation was then thoroughly irrigated with warm, sterile salt solution, except in the cases of dogs II and III, in which irrigation with bichloride (1:1000) preceded the use of the salt solution; the muscles were brought together with deep catgut sutures and the skin wound was closed with a continuous silk suture; the wound was painted with collodion and dressed with iodoform gauze and bandaged. In none of the cases did suppuration occur.

Test for regeneration.—No antiseptic precautions were observed. The dog was given a hypodermic injection of 0.05 gramme of the sulphate of morphia, and etherized; the trachea and carotid artery were connected with a tambour and a mercury manometer respectively, and these in turn were arranged to record upon a kymographion. A time recorder and a stimulating key also wrote upon the kymographion, so that the kymographic tracings were made to show four simultaneous records, namely, of the circulation, respiration, time, and duration of the stimulation.

The nerve, ganglion and roots were exposed as in the first operation, but greater difficulty was encountered owing to the presence of a considerable amount of scar tissue. In dogs A, I and II, the entire nerve was exposed before stimulating; in dogs B, III, IV, V, VI and VII, stimulations were made as the dissection advanced towards the ganglion.

The electrical stimulations were faradic and of varying strength; weak, moderate and strong. A weak stimulus was a current just perceptible on touching the electrodes to the tongue; a strong stimulus was a current painful to the tongue, and a moderate stimulus was of intermediate strength. Care was taken at each stimulation to prevent radiation through the surrounding tissues. In some cases mechanical stimulation was employed, which consisted in crushing the nerve with forceps, or in tightly ligating it.

After the desired data had been obtained, the animal was killed; the vertebral canal and cranial cavity were laid open with bone forceps; the brain and spinal cord, as far caudally as the sixth cervical nerve, were removed for histological examination. Great care was taken not to injure the brain and cord. The right second cervical nerve with its ganglion and roots was also removed for histological examination.

For the sake of clearness and convenience the results of the kymographic tracings have been tabulated (Table I). The table does not include the results of every stimulation, for where several stimuli in the same region gave identical results only one or two have been taken as examples. Also, all results have been discarded where it seemed possible that they were influenced by any accidents, such as bad electrodes, imperfect isolation of the nerve, etc. The results obtained with the two normal dogs (A and B) are so nearly identical that it has been considered sufficient to insert a single record (Dog A).

EXPLANATION OF TABLE I.

The figures in the column on the extreme left give the order of sequence of the stimuli. The second column states the part of the nerve stimulated. The third column gives the greatest amplitude of the respiratory curve in millimetres: (1) during the period of 10 seconds preceding stimulation, (2) during the period of stimulation, (3) during the period of 10 seconds immediately following the stimulation, and (4) during the succeeding period of 10 seconds. The fourth column gives the rate of respiration per 30 seconds during the same periods of time.

As it is often impossible to appreciate the relative values of small variations in the blood pressure, the approximate *average size* of the pulse waves for the period of 10 seconds preceding stimulation is given in column five. In columns six and seven the blood pressure in millimetres of mercury and the pulse-rate per 30 seconds have been recorded in the same manner and for the same periods as with the respiration. Columns eight, nine and ten state some of the characteristics of the stimuli. Sometimes it was impossible to determine the duration of a mechanical stimulus, and, in such cases, four periods, each of ten seconds duration, were taken in such a manner that stimulation begins with the first second of the second period. Such a procedure is denoted by an asterisk (*) in column ten. In column eleven the results of the stimulations are summed up: "positive" indicating that the stimulation produced reflex changes similar to those obtained with a normal dog; "negative" meaning that there were no appreciable reflexes.

From inspection of the table it appears that a more or less complete return of function occurred in every case, although in dogs IV and VI the reflex effects were very slight. Such an apparent return of function might be accounted for in several ways:

(1) *Imperfect destruction of continuity in the first operation.*— The possibility of this source of error is removed by the following experiments. After obtaining the usual effects of stimulation of the second cervical nerve of a normal dog (Dog B), the roots were ligated in the usual manner. Subsequent stimulation produced no effect whatever. Again in the case of Dog III, after giving the routine stimulations, the roots were ligated in the usual manner. Subsequent stimulations were without effect, and microscopical examination of the nerve showed a complete break in the continuity of the fibres at the point of ligation.

TABLE I.—Dog A. (Normal.)

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Amplitude of Pulse wave.	Circulation. Blood pressure.	Circulation. Rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis magnus.*	5 41 44 11	7 50 39 22	36	130 145 148 123	47 55 45 44	Electrical.	Moderate.	7 sec.	Positive.
2	Base of skull.	14 47 41 20	16 40 39 35	24	124 148 150 126	52 64 56 48	do.	do.	5 sec.	do.
3	2 mm. from ganglion.	4 34 32 2	6 53 68 11	30	120 150 151 131	36 57 49 37	do.	do.	4 sec.	do.
5		3 13 12 3	10 53 52 32	18	121 140 156 100	52 65 56 48	do.	Strong	7 sec.	do.

Dog 1.

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Amplitude of Pulse wave.	Circulation. Blood pressure.	Circulation. Rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis magnus.*	2 31 32 10	.. 52 41 47	54	136 176 136 120	40 55 18 51	Electrical.	Weak	5.2 sec.	Positive.
2	Top of skull.	3 4 1 ..	31 25 24 ..	38	131 122 125 ..	45 43 43 ..	do.	do.	4.3 sec.	do.
3	Base of skull.	4 13 9 10	24 37 37 29	38	125 110 128 124	42 47 42 14	do.	do.	9.1 sec.	do.
5		10 32 13 10	21 40 48 48	30	122 138 132 118	48 56 49 51	do.	Strong	8.4 sec.	do.
6	15 mm. from ganglion.	7 9 9 9	27 30 27 29	26	131 132 128 127	53 52 17 52	do.	Weak	8.9 sec.	do.
7		8 10 9 6	26 29 28 21	24	126 132 128 126	50 50 48 48	do.	do.	9.3 sec.	do.
8		5 11 10 9	23 30 30 28	30	126 134 128 129	49 50 47 50	do.	Strong	9.4 sec.	do.

TABLE I.—*Continued.* Dog II.

No.	Part of nerve stimulated.	RESPIRATION. Amplitude.	RESPIRATION. Rate.	CIRCULATION. Amplitude of pulse wave.	CIRCULATION. Blood pressure.	CIRCULATION. Pulse rate.	STIMULUS. Kind.	STIMULUS. Strength.	STIMULUS. Duration.	Result.
13	*Occipitalis magnus.*	0 2 2 2	6 7 7 7	20	130 128 130 132	39 39 38 37	Electrical.	Moderate.	12 sec.	Negative.
14	Base of skull.	2 2 2 2	7 7 6 7	24	128 126 126 127	37 37 36 36	do.	do.	14 sec.	do.
11	3 mm. from ganglion.	0 3 3 2	9 8 6 3	14	106 109 114 117	45 45 45 45	do.	do.	12 sec.	do.
10	*Ganglion.*	3 13 16 10	8 25 34 18	16	122 131 132 110	42 41 45 47	do.	do.	12 sec.	Positive.
17		1 19 19 10	5 31 15 9	20	104 115 116 107	41 41 44 45	do.	do.	8 sec.	do.
18		2 22 22 12	8 39 24 8	16	114 124 106 114	41 42 41 40	do.	do.	8 sec.	do.
16	*Roots.*	2 15 15 6	7 23 12 5	20	116 124 122 114	39 39 40 41	do.	do.	10 sec.	do.

Dog III.

No.	Part of nerve stimulated.	RESPIRATION. Amplitude.	RESPIRATION. Rate.	CIRCULATION. Amplitude of pulse wave.	CIRCULATION. Blood pressure.	CIRCULATION. Pulse rate.	STIMULUS. Kind.	STIMULUS. Strength.	STIMULUS. Duration.	Result.
1	*Occipitalis magnus.*	1 1 2 2	6 6 7 7	22	126 121 120 119	59 53 53 52	Electrical.	Weak.	10 sec.	Negative.
2	Top of skull.	3 2 1 2	7 7 7 9	22	120 120 120 123	54 52 51 51	do.	Strong.	18 sec.	do.
19	25 mm. from ganglion.	15 28	42 47	7	115 166 142 100	90 84 89 95	Mechanic'l	Crush.	9 sec.	Positive.
21	15 mm. from ganglion.	11 36 30 18	19 44 48 52	7	126 164 110 94	89 .. 91 94	do.	do.	8 sec.	do.
6	4 mm. from ganglion.	4 3 4 4	12 12 12 12	18	95 97 97 97	57 60 55 57	Electrical.	Weak.	8 sec.	Negative.
8		4 4 5 5	11 12 12 12	14	103 107 106 106	66 68 69 67	do.	Strong.	7 sec.	.. ?
7	*Ganglion.*	4 4 5 5	11 12 12 12	20	96 97 97 97	59 60 60 62	do.	Weak.	10 sec.	Negative.
9		5 12 12 8	11 23 37 30	14	104 118 124 114	67 76 79 81	do.	Strong.	8 sec.	Positive.
10		4 22 15 4	19 40 36 41	8	118 123 120 102	89 87 87 92	do.	do.	5 sec.	do.

TABLE I.—*Continued.* Dog IV.

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Circulation. Amplitude of Pulse wave.	Circulation. Blood pressure.	Circulation. Pulse rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis*	1 2 3 3	8 8 12 8	14	98 104 103 97	67 57 56 57	Electrical.	Weak.	5 sec.	Positive.
2	*magnus.*	3 2 4 3	8 9 9 11	14	100 105 106 111	53 59 51 51	do.	Strong.	6 sec.	do. ?
3	Base of skull.	2 2 1 1	13 14 10 11	16	104 108 109 106	53 53 49 51	Mechani'l.	Ligation.	10* sec.	do. ?
8	2 mm. from	1 1 1 1	17 17 15 16	7	56 58 58 58	98 96 95 96	Electrical.	Strong.	8 sec.	Negative.
19	ganglion.	1 1 1 1	18 17 18 16	6	63 61 61 60	89 90 90 90	do.	do.	7 sec.	do.
12	*Ganglion.*	1 1 1 1	16 18 19 18	6	58 57 59 61	94 95 93 95	Mechani'l.	Crush.	6 sec.	do.

Dog V.

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Circulation. Amplitude of Pulse wave.	Circulation. Blood pressure.	Circulation. Pulse rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis*	1 1 1 1	32 32 33 30	38	132 133 132 133	35 35 38 33	Electrical.	Weak.	8 sec.	Negative.
2	*magnus.*	1 1 1 1	30 30 30 31	34	133 135 134 134	33 30 36 36	do.	Strong.	9 sec.	do.
3	Top of skull.	2 3 3 3	31 36 48 45	36	136 142 134 122	35 41 48 50	Mechani'l.	Crushing.	10 sec.	Positive.
4		3 8 14 4	45 48 57 52	20	122 142 130 ..	50 53 66 ..	do.	Ligation.	10* sec.	do.
5		1 1 1 1	26 28 25 24	64	154 155 154 153	31 32 32 30	Electrical.	Weak.	12 sec.	Negative.
6	Base of skull.	1 2 2 1	24 32 36 33	60	154 155 146 144	30 34 36 34	do.	Strong.	10 sec.	Positive.
9		1 1 1 1	23 21 25 23	56	143 140 140 140	30 31 30 30	Mechani'l.	Crush.	11 sec.	Negative.
18	2 mm. from	4 3 4 4	20 22 20 20	5	98 92 96 94	83 80 83 81	Electrical.	Strong.	11 sec.	Positive.
19	ganglion.	2 2 4 4	19 22 19 21	6	91 93 94 98	82 76 79 80	do.	do.	9 sec.	do.
17	*Ganglion.*	3 10 11 4	19 57 18 20	5	100 103 80 86	85 85 84 83	do.	do.	7 sec.	do.
20		4 19 11 3	19 62 21 22	5	94 106 84 86	81 79 81 83	do.	do.	6 sec.	do.
25	*Roots.*	2 2 2 2	18 20 19 19	4	94 94 95 95	67 67 68 69	do.	Moderate.	9 sec.	Negative.

TABLE I.—*Continued.* Dog VI.

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Circulation. Amplitude of pulse wave.	Circulation. Blood pressure.	Circulation. Pulse rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis*	2 2 2 2	15 17 17 17	14	118 119 119 119	60 59 59 59	Electrical.	Weak.	18 sec.	Negative.
3	*magnus.*	2 2 2 1	17 17 15 17	14	123 124 122 122	58 58 59 60	do.	Strong.	8 sec.	do.
4	Top of skull.	1 1 1 1	17 18 17 18	14	126 126 128 125	54 59 56 56	Mechani'l.	Crushing.	10 sec.	do.
7	2 mm. from	3 4 3 4	15 15 15 15	8	100 101 98 102	65 65 67 69	Electrical.	Strong.	5 sec.	do.
14	ganglion.	3 4 4 5	19 18 12 18	3	103 99 99 106	101. 100 92 92	do.	do.	13 sec.	Positive.
15		4 4 4 3	18 18 19 18	3	107 107 107 107	96 98 93 95	Mechani'l.	Crushing.	10* sec.	Negative.
8	*Ganglion.*	4 4 4 3	15 17 17 15	5	102 97 106 106	78 78 81 71	Electrical.	Weak.	10 sec.	Positive.
13		4 4 4 4	20 20 20 18	3	101 92 100 103	100 98 98 99	do.	Strong.	16 sec.	do.
18	*Roots.*	6 4 6 7	15 12 14 13	2	111 111 106 114	102 90 89 85	do.	Weak.	14 sec.	do.
19		7 4 5 6	20 16 16 15	2	114 103 113 115	90 91 96 84	do.	do.	13 sec.	do.

Dog VII.

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Circulation. Amplitude of pulse wave.	Circulation. Blood pressure.	Circulation. Pulse rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis*	10 9 7.5 6	30 27 28 25	2	169 169 175 173	75 72 75 76	Electrical.	Weak.	14 sec.	Negative.
2	*magnus.*	7 7 8 6	21 22 23 21	2	172 173 171 173	76 78 73 73	do.	Strong.	14 sec.	do.
3	Base of skull.	6 7 6 ..	23 18 18 ..	2	173 174 174 ..	75 71 70 ..	*Mechanical*	*Forceps.*	14 sec.	do.
5	2 cm. from ganglion.	8 14 14 12	12 17 19 18	2	140 152 156 152	93 92.1 93 96	Electrical.	Weak.	14 sec.	Positive.
12	1 cm. from	1 28 16 0	13 54 39 ..	1	156 184 118 146	117 117	do.	do.	47 sec.	do.
7	ganglion.	13 32 46 33	9 74 43 38	1	142 146 144 138	.. ..	do.	do.	9 sec.	do.
10	*Ganglion.*	3 33 38 29	12 70 45 34	1	149 172 122 127	.. ..	do.	do.	12 sec.	do.
11		1 27 23 0	13 71 24 0	1	142 181 112 131	117 .. 115 117	do.	do.	7 sec.	do.

(2) *Anastomosis with neighboring nerves.*—The above mentioned experiments on Dogs B and III also show that although the finer ramifications of the second cervical anastomose with the third cervical and with the accessorius, still, it is not possible for impulses to pass from the second cervical nerve to the cord through either of these connections.

(3) *Radiation to the cord through the surrounding tissues.*—This also appears highly improbable, for in every case the nerve was dissected out and retracted from the surrounding tissues before the electrodes were applied; and in the case of the ganglion, where such retraction was impossible, many of the stimulations especially with strong currents were accompanied by control stimulations of the surrounding tissues, and always with negative results.

(4) *Transmission of the impulses through the dorsal root of the nerve stimulated.*—This is considered the only possible explanation for the results obtained. Such a return of function in the dorsal roots can occur only in two ways. The root may be regenerated by fibres having their trophic centres within the cord; or secondly, by fibres having their trophic centres outside the cord. It is the usually accepted view that no sensory fibres are derived from cells within the cord, nevetheless it seems advisable to call attention to the work which goes to prove that the dorsal root of the nerve in question does not contain such fibres. This will be especially useful for reference in the section on histology.

In the chick of four to eleven days' incubation, it has been abundantly proved by von Lenhossék (12), Cajal (13), van Gehuchten (14) and Retzius, that fibres arising from a group of cells situated in the ventral horn, after running dorsally and entering the dorsal root, pass directly through the root ganglion without communicating in any way with the ganglion cells. Kölliker (15) is of the opinion that they are vaso- and viscero-motor fibres on their way to the sympathetic system. Up to the present time, however, no observer has been able to demonstrate microscopically the occurrence of such fibres in the mammalia.

According to the original observations of Waller (16) the trophic centres of all the fibres of the dorsal root are the spinal ganglion cells. Vejas (17) and Joseph (18) stated that they found undegenerated fibres

in the proximal part of sectioned dorsal roots, but no one has been able to confirm their observations. On the contrary, Waller's experiments have been repeated and his results confirmed by Bernard (19), Kahler (20), and more recently by Singer and Münzer (21). Sherrington (22) observed such "undegenerated fibres" in the cat, but he gives excellent reasons for believing that they are "embryonic fibres" (23) and not undegenerated fibres.

It might be claimed that splanchnic efferent fibres occur in the dorsal root (24, 25) of the second cervical nerve, as is probably the case with some of the lumbar and sacral nerves. It has been demonstrated, however, by Gaskell (26) and Langley (27) that the splanchnic efferent fibres traverse the white and not the gray rami communicantes. They have also shown that the second cervical nerve in the dog is connected with the sympathetic system (sup. cerv. gang.), not by a mixed ramus communicans, but by a gray ramus alone (28), the rami viscerales of the upper cervical nerves (as well as of the vagus) being represented by the internal branch of the accessorius.

With regard to the occurrence in the second cervical nerve of trophic fibres to the skin, it may be stated that although ligation was performed on both roots of the nerve in seven dogs, no trophic disturbances resulted in the area supplied by the nerve such as Joseph (29) has described in the cat.

Such fibres, moreover, whether splanchnic-efferent or trophic, would not give reflex effects on stimulation, inasmuch as they would be efferent fibres, and the fact that they probably do not exist is therefore of importance only in relation to the subsequent histological study of the cord and nerve.

Hence it is a justifiable conclusion that this dorsal root contains only such fibres as have their trophic centres situated outside the cord, and consequently the presence of root fibres on the central side of the point at which the continuity of the fibres has been destroyed is proof of a regeneration from the ganglion to the cord, and any return of function is a proof of regeneration of those fibres, both in the root and within the cord itself.

As has been said, a more or less complete regeneration occurred in every case. The character of the regained functional activity varied considerably in the different cases. In some the respiratory effects preponderated, in others the circulatory.

As a commentary to the tables and as a means of calling attention to numerous interesting facts shown by them, the following summary of results is given:

Dog. A (normal). The *amplitude* and *rate of respiration, blood pressure,* and *pulse rate* were in all cases markedly *increased* by stimulation of the second cervical nerve.

Dog I. The *increase* in the *amplitude* of the respiratory curve was as great as in the normal dog (Dog A). The effect on the *rate of respiration* was *variable,* but usually an acceleration was observed. There was a *rise of blood pressure* during the period of stimulation and the *pulse rate* was, in general, *accelerated,* especially during the earlier stimulations.

Dog II. The respiration was but little affected by stimulation of the peripheral nerve, but stimulation of the ganglion and roots caused about as great an *increase* in the *amplitude* as in Dog A, while the *acceleration of rate,* although marked, was proportionally less than in A. Stimulation at the periphery caused very little change in the *blood pressure;* nearer the ganglion a slight *rise,* while at the ganglion and roots the *rise* was pronounced. The *pulse rate* showed little or *no variation.*

Dog III. A *rise* of *blood pressure* occurred with mechanical stimulation of the peripheral nerve and with strong electrical stimulation at and near the ganglion (No. 8), the last being the least marked. Other stimulations caused no variation in blood pressure. The effect on the *pulse rate* was *inconstant.* The *amplitude* and *rate of respiration increased* with the blood pressure, except in case of No. 8, in which no variation occurred.

Dog IV. The *respiration* remained throughout *unaffected.* The *blood pressure* was *slightly raised* by the first three stimulations, after which stimulation produced no effect. The *pulse rate* was altered in the first three cases only. These were stimulations of the peripheral nerve, of which two caused a *slowing* and one an *acceleration* of the pulse rate.

Dog V. The *rate* of respiration was *increased* by mechanical stimulation of the peripheral nerve near its distal end, strong electrical stimulation of the same at the base of the skull, and strong electrical stimulation at the ganglion. In the two last cases the *amplitude,* which elsewhere remained unaffected, was *increased.* The *blood pressure* varied under the same conditions as the rate of respiration, this variation consisting in a *fall* or less frequently a *rise.* The *pulse rate* was affected (*accelerated*) only in the cases of mechanical and strong electrical stimulation of the peripheral nerve.

Dog. VI. The *respiration* remained *unchanged,* except in No. 14. The *blood pressure* was not appreciably influenced by stimulation of the peripheral nerve except in one case (No. 14), but stimulation of the ganglion and roots caused a *fall followed by a rise* to or above the normal. There was a *slowing* of the *pulse* in two cases (Nos. 14 and 18), but otherwise no change occurred.

Dog VII. Stimulation of the more peripheral part of the nerve produced no effect. Stimulation of the nerve near the ganglion, of the ganglion itself and of the roots, caused a marked increase in the *amplitude* and *rate* of respiration. The rise of *blood pressure* was in some cases very marked and usually followed by a fall. The counting of the pulse rate was prevented by the fact that the great oscillations in pressure caused by the violent respirations obscured the pulse record.

The condition of the nerve trunk peripheral to the ganglion was in several instances abnormal, especially in the more peripheral parts of its course. (1) It was in some cases of a dull, opaque, grayish-white appearance (Dogs II, III and VI), and in one case (Dog VI) the ganglion was swollen, elongated and bound down by adhesions. (2) It often responded, not to electrical, but only to mechanical stimuli (Dog III, Nos. 19, 20; Dog V, Nos. 3, 4). (3) It often responded only to the first few stimulations, soon losing its irritability (Dog V, Nos. 3, 4, 9).

All these facts point to a recent regeneration of the peripheral nerve (30). The primary degeneration in the nerve trunk peripheral to the ganglion, which this implies, was considered to be due to the contraction of the scar tissue following the first operation, for this scar tissue, as has been stated, was often quite abundant in the region of the old wound.

When the stimulus was applied to the nerve peripherally to the ganglion, it was often impossible to distinguish between the effects due to recent regeneration of the peripheral nerve and those due to recent regeneration of the root. If, however, a comparison be made of these results with those obtained by stimulating the ganglion or roots, there will be no chance of confusing results due to peripheral with those due to more central regeneration. The condition of the peripheral nerve and of the root, their reaction to stimuli and certain other data of interest and importance are stated in the following table (Table II).

TABLE II.

Dog.	Approximate age.	Intervals between first and second operation.	Scar tissue found at second operation.	Appearance of peripheral nerve.	RESULTS OF STIMULATION.			
					Peripheral.	Nearer ganglion.	Ganglion.	Roots.
		Days.						
I.	Adult.	90	Much.	Normal.	+ e	+ e	..	..
II.	About 6 mo.	92	Much.	Abnormal.	— e	— e	+ e	+ e
III.	About 6 mo.	88	Much.	Abnormal.	+ m, — e	— e	+ e	..
IV.	Adult.	90	Moderate.	Normal.	— m, — e	— m, — e	— e	— e
V.	Young adult.	89	Moderate.	Normal.	+ m, — e	— m (later)	+ e	— e (later)
VI.	About 6 mo.	151	Moderate.	Abnormal.	— m, — e	— m, — e	+ e	+ e
VII.	About 6 mo.	109	Much.	Normal.	— m, — e	+ e	+ e	+ e

e = with electrical stimuli.

m = with mechanical stimuli.

— = negative results.

\+ = positive results. For example: + e = positive results with electrical stimuli.

From the results of the experiments described above we feel justified in concluding, that *after severance of the fibres of the dorsal root of the spinal nerves between the ganglion and the cord, regeneration of the fibres into the cord will take place under proper conditions, so that normal reflexes through the respiratory, cardiac and vasomotor centres may be obtained.*

As to the completeness of the regeneration and the average time necessary for the restoration of function, it is not possible for us to speak positively, owing to the small number of our experiments. The results of the seven experiments indicate that there may be great individual differences in the rapidity of regeneration. In some cases the return of functional activity in the dorsal root fibres seemed to be nearly complete at the end of 90 days, while in one case the return was far from complete after an interval of 151 days.

We may add in conclusion that if the posterior root fibres can thus be regenerated in the posterior columns of the cord, there seems reason to hope that the fibres in other tracts may possess the same property, and that therefore it is not impossible that with the proper technique a severed spinal cord might be made to regenerate its broken tracts both ascending and descending.

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ACTION OF FORMALDEHYDE ON ENZYMES AND ON CERTAIN PROTEIDS.

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It is a well known fact that formaldehyde, even in very small amount, will exert a peculiar action on proteid material, hardening it or otherwise altering its chemical and physical properties. Fibrin, when allowed to stand in a solution of formaldehyde even as dilute as 1:500, soon becomes hardened and resists, as will be presently shown, the action of proteolytic enzymes, and although it may finally be digested the process is nevertheless greatly retarded. Again, egg albumen or blood serum to which a small amount of formaldehyde has been added will not coagulate when heated; and numerous other instances might be cited showing the changes that take place when formaldehyde is present.

Formaldehyde also has powerful germicidal properties, and even in small amount will inhibit the growth of micro-organisms. In stronger concentration it will promptly destroy bacteria.

The exact chemical nature of the soluble ferments, or enzymes, is far from being understood. Some authorities claim they are proteid-like substances. When obtained in the purest condition possible they contain nitrogen, and many give some of the proteid reactions. Certain of the purified ferments, however, fail to give the common proteid reactions. When such reactions are given by a ferment presumably pure, it is still impossible to determine whether these are due to contamination with proteid material, or whether they are due to the ferment itself.

The experiments to be described were carried on with a view of determining whether formaldehyde exerts any action on some of the more common soluble ferments, especially those concerned in the processes of digestion. Accepting the common view that enzymes are

proteid compounds, it might be expected that they would undergo alteration in the same way as ordinary proteid substances. Such change in the composition of the enzyme would be shown by a decreased or wholly suppressed ferment action. It should be borne in mind, however, that a decrease in ferment action, or its entire absence, may be due to the action of the formaldehyde on the substance to be digested. Thus, fibrin which has been kept in a solution of formaldehyde 1:100 for a day is scarcely affected by a solution of pepsin-hydrochloric acid, even after being kept at a temperature of about 38° for several hours. On the other hand, formaldehyde may be added to the digestive fluid in the proportion of 1:100, and at the end of several weeks this solution will be found to be still active; in fact, it will dissolve fresh fibrin nearly if not quite as rapidly as a fresh solution of pepsin-hydrochloric acid.

The literature contains but very little concerning the effect of formaldehyde on digestive ferments. According to Loew,* pepsin and disastase lose their activity when left in contact with formaldehyde for one day; the amounts of substances used were 1 gramme of ferment, 10 grammes of water, and 5 cc. of 15 per cent formaldehyde solution (=5 per cent formaldehyde in the mixture). Other ferments, emulsin, papaïn, tryspin, used in the form of crude products, soon give, like many other proteid substances, precipitates with formaldehyde which are very difficultly soluble in acids and in alkalis. Simons † found that formaldehyde has no apparent effect on peptic digestion, but has a very depressing action on the pancreatic ferment (trypsin), even one part in 2000 parts of the solution being sufficient to distinctly retard digestion. We have been able to confirm this statement. Maybery and Goldsmith ‡ tried the action of several antiseptics on peptic digestion. Their results show that the greater the amount of formaldehyde used the greater will be the percentage of fibrin undigested in a given time. In Wurtz's Dictionnaire de Chimie, Deuxième Supplément, vol. iii, p. 313, the action of formaldehyde on proteid material and on ferments is considered very briefly.

* *Journ. f. prakt. Chem.*, 1888, xxxvii, 101.
† *J. Am. Chem. Soc.*, 1897, xix, 744.
‡ *J. Am. Chem. Soc.*, 1897, xix, 889.

The statement is made that the soluble ferments (diastase, pepsin, pancreatin, etc.) are modified completely by formaldehyde, when this is used in solution of sufficient concentration. This statement, as will be presently shown, does not hold true for pepsin, malt diastase and rennin.

In view of the rather incomplete knowledge in regard to the action of formaldehyde on digestive ferments, it was deemed advisable to make a systematic study of the subject. The ferments pepsin, rennin, pancreatin and papaïn were used; these were ordinary commercial preparations, and were found by control experiments to be more or less active. The pancreatin, however, possessed very little diastatic power, and consequently an aqueous extract of fresh pancreatic gland was used for the purpose of observing the effects on the diastatic as well as on the tryptic ferments. The commercial preparations of ptyalin and malt diastase were found to possess but very little activity, and hence saliva and malt were employed instead. Inasmuch as the preparations referred to are far from being pure ferments and contain more or less foreign material, it is obvious that the ratio between the amounts of formaldehyde and ferment cannot be expressed. All that can be given are the amounts of materials used, the dilution, the amount of formaldehyde, the conditions, and the results obtained. In all the tests the conditions most suitable to the normal action of the ferments, *i. e.* temperature, reaction, etc., were adhered to as closely as possible. When ferments having similar properties were compared, the tests were made under conditions as nearly alike as possible. Several series of tests were made with each ferment; the conditions were varied somewhat with each series, as will be seen from the detailed experiments. When doubtful results were obtained, the tests were repeated. In general, the work was carried on in the following manner: A certain amount of the commercial ferment, or of the solution of the ferment, was dissolved in distilled water, and this solution was divided into portions; to each a definite amount of "formalin" was added, so that the liquid contained known amounts of formaldehyde, varying usually from 1:100 to 1:000. These mixtures of formaldehyde and ferment were allowed to stand in corked

flasks at ordinary room temperature. From time to time portions were tested in order to determine whether the activity had been affected, and if so to what extent. The formalin used was approximately a 40 per cent solution, as determined by analysis; it contained only a trace of free acid, too small to be estimated. Fresh, well washed fibrin from the blood of steers was used in testing the proteolytic ferments, and freshly prepared one or two per cent starch paste, made of potato starch, for the diastatic experiments. The experiments were carried on at a temperature of 37°-40°, except in the case of malt diastase, where the temperature of 57°-60° was employed.

ACTION OF FORMALDEHYDE ON FIBRIN.

Benedicenti * gives a review of the work that has been done on the action of formaldehyde on various proteid substances, and also the results of several series of experiments carried on by himself. He confirms the statements that had been made, that formalhehyde hardens proteids and renders them incapable of swelling in dilute hydrochloric acid, and of being digested by pepsin-hydrochloric acid or by pancreatin juice.

Several series of tests were made in order to determine how much formaldebyde must be used and for how long a time it must be allowed to act in order that fibrin would become so altered that it would be incapable of being digested.

Experiment I.—A few small shreds of fibrin were added to portions of 15 cc. each of an active solution of pepsin. Formaldehyde was then added in the proportions of 1:100, 1:250, 1:1000, and 1:2500. The tubes were corked and set aside for 24 hours at ordinary temperature. An equal volume of 0.5 per cent hydrochloric acid was then added to each tube and the tubes were placed in a water-bath maintained at a temperature of 38°-40°, and examined at intervals during the next 24 hours. Table I shows the changes that took place. Tube C served as a control.

In this experiment the fibrin and pepsin were both subjected to the action of the formaldehyde. The fibrin alone was affected.

Experiment II.—Fibrin was allowed to stand in solutions of formaldehyde 1:500 and 1:1000 at ordinary temperature. At the end of three

* *Archiv f. Anat. u. Physiol.* (Physiol. Abtheilung), 1897, p. 219.

days small pieces were removed and squeezed thoroughly, then subjected to the action of a stronger solution of pepsin-hydrochloric acid than that used in the preceding experiment. At the end of three-quarters of an hour the fibrin that had been exposed to the weaker solution of formaldehyde was dissolved almost completely, and disappeared entirely a few minutes later. That which had been exposed to the stronger solution of formaldehyde dissolved more slowly. At the end of an hour it was swollen, and the liquid was slightly cloudy; after another hour it had entirely dissolved.

TABLE I.

Mixtures of Pepsin, Fibrin and Formaldehyde—24 Hours at Ordinary Temperature, then Digested at 38°–40°.

Tube No.	Formaldehyde.	Examined at the end of 1 hour.	2½ hours.	4 hours.	24 hours.
1.	1:100	No change.	No change.	Fibrin slightly swollen; liquid clear.	Fibrin nearly dissolved.
2.	1:250	Fibrin slightly swollen; liquid clear.	Fibrin dissolving slowly; liquid cloudy.	Fibrin dissolving slowly.	Fibrin nearly dissolved.
3.	1:1000	Fibrin swollen; liquid cloudy.	Fibrin nearly dissolved.	Fibrin dissolved.	
4.	1:2500	Fibrin dissolving; liquid cloudy.	Fibrin almost dissolved.	Fibrin dissolved.	
C.	......	Fibrin dissolved.			

Similar results were obtained with an aqueous extract of pancreatic gland. The solution used was the one described in Experiment XVIII. Fibrin, which had been exposed to the action of formaldehyde 1:1000 for 24 hours at room temperature, was digested nearly as rapidly as fibrin which had not been exposed to formaldehyde; that which had stood in formaldehyde 1:500 for the same length of time was digested more slowly, though it did not dissolve completely.

Fibrin, which had stood in formaldehyde 1:1000 for 24 hours at ordinary temperature, or for only a few hours at 40°, was digested completely by the commercial pancreatin, though much more slowly than fresh fibrin. When, however, the action of formaldehyde had been continued for a few hours longer at 40°, the pancreatin had no effect.

The results with papaïn will be described later. It might be said here, however, that a strong solution of the ferment had no effect on fibrin that had been exposed to formaldehyde 1:1000 for a few hours at room temperature, or even for half an hour at 40°.

These results show that formaldehyde, even in very small amount, will alter fibrin in the course of a few hours so that it will offer considerable resistance to the action of proteolytic ferments. The action of the formaldehyde is more marked at a temperature of 40° than at ordinary room temperature. Although the fibrin may be digested the process is retarded. Peptic digestion was affected the least.

ACTION OF FORMALDEHYDE ON MILK.

Pottevin* observed that formaldehyde retards the coagulation of milk by rennet, and that rennet and sucrase on contact with strong solutions of formaldehyde become inactive. He also found that inversion of sugar by sucrase or even by acids was retarded or suppressed by formaldehyde. Weigle and Merkel † found that the precipitate of casein in milk containing formaldehyde is coarsely flocculent and voluminous, and that the digestion of milk and egg albumen is prevented by formaldehyde. The action of formaldehyde on the proteids of milk, as well as on other proteid substances, has been studied by numerous other investigators.

Milk is not coagulated by formaldehyde. If formaldehyde is added to milk in the proportion of 1:500, the latter will be altered within a few hours so that it will not be coagulated by an active solution of rennin. If less formaldehyde is added coagulation will take place, but slowly.

The rennet used was Liquid Rennet, made by John Wyeth and Bro. When diluted with four parts of water (1-4), 1 cc. of this dilute rennet added to 10 cc. of fresh milk and kept at 40° would give a solid coagulum in about three-quarters of an hour to an hour. When diluted with an equal volume of water (1-1), 1 cc. would coagulate 10 cc. of milk in about ten minutes, or if the milk were previously warmed to 40°, in two or three minutes.

Experiment III.—Formalin was added to portions of 240 cc. of fresh milk in flasks in the following proportions:

Flask	1	received	24	cc. formalin (40 per cent) = formaldehyde	1:25
"	2	"	6	" "	1:100
"	3	"	1.2	" "	1:500
"	4	"	.6	" "	1:1000
"	C	"	no formalin and served as a control. Fresh milk was always used in the control tests.		

* *Ann. Inst. Pasteur*, 1894, viii, 796.

† Forschungsberichte über Lebensmittel. München, 1895, pp. 91-94.

The flasks containing the milk and formaldehyde were corked and set aside in a cool place. At stated intervals portions of 10 cc. were taken out of each flask, 1 cc. of the dilute rennet (1-4) added, and the tubes then placed in the water-bath and kept at 38°-40°. If no coagulation took place within 24 hours they were reported negative. Three sets of tests were made, extending over a period of four days. Table II contains the results.

TABLE II.

Tube No.	Formaldehyde.	Mixtures of Milk and Formaldehyde tested at the end of 4½ hours.	36 hours.	4 days.
1..	1:25	No coagulation.	No coagulation.	No coagulation.
2..	1:100	"	"	"
3..	1:500	"	"	"
4..	1:1000	Prompt coagulation.	Prompt coagulation.	Milk thickened in about two hours and became solid later.
C..	....	"	"	Prompt coagulation.

Experiment IV.—Another series was made, using as before portions of 240 cc. of milk to which formalin had been added in the following proportions:

Flask 1 received 1.2 cc. formalin (40 per cent) = formaldehyde..............1:500
" 2 " 0.8 " " 1:750
" 3 " 0.6 " " 1:1000
" 4 " 0.4 " " 1:1500
" C " no formalin and served as a control.

The tests were made in the same way as those just described, using, however, a stronger ferment solution (1-1), which moreover contained formaldehyde in the proportion of 1:500. The same mixture was used for all three series of tests. As will be explained later, the ferment is not affected by very small amounts of formaldehyde, and although the formaldehyde will rapidly alter the casein of the milk, coagulation will result too rapidly for this effect to be of any influence. In the series of tests made on the 4th day the milk was warmed to 40° before use. The results were as follows (Table III):

TABLE III.

Tube.	Formaldehyde.	Mixtures of Milk and Formaldehyde tested at the end of 1 hour.	24 hours.	4 days.
1..	1:500	Thick in 1¼ hours, solid soon after.	No coagulation.	No coagulation.
2..	1:750	Thick in 15 min., solid in 30 min.	Thick in 20 min., solid in 40 min.	No coagulation even after 24 hours.
3..	1:100	Coagulation in about 15 min.	Thick in 20 min., solid in 40 min.	Thick in 1¼ hours, solid soon after.
4..	1:100	Coagulation in 15 min.	Coagulation in 20 min.	Thick in 15 min., solid in 20 min.
C..	....	Coagulation in about 15 min.	Coagulation in 10 min.	Coagulation in 3-4 min.

These results show that milk to which formaldehyde has been added in considerable quantity undergoes alteration. The casein is rendered incapable of being coagulated by rennet ferment. If formalin is added in smaller amount (1:1000 formaldehyde), the casein is acted upon more slowly and will be coagulated slowly or not at all. The action of formaldehyde on rennet ferment will be considered later.

Several tests were made to determine whether the coagula produced by rennet in milk to which formaldehyde had been added would be digested by pepsin-hydrochloric acid. It was found that the casein behaved just as the fibrin had, that is, if very little formaldehyde had been used the process was simply retarded, while if formaldehyde had been added in larger amount digestion would fail to take place.

It is evident from the preceding experiments that a 1 per cent solution of formaldehyde acting on fibrin for 24 hours will render this practically insoluble in pepsin-hydrochloric acid. Furthermore, the addition of formalin to milk alters the casein to such an extent that this will not be precipitated, or but slowly on subsequent addition of rennet ferment.

ACTION OF FORMALDEHYDE ON PEPSIN.

The pepsin employed (Kahlbaum's) was a white powder, very easily and completely soluble in water. One gramme in 1000 cc. of 0.25 per cent hydrochloric acid formed a very active digestive fluid, as will be seen from the rapidity with which fibrin was dissolved. One series of experiments was made in which formaldehyde was added to portions of a solution of pepsin-hydrochloric acid. In another series the formaldehyde was added in the same amounts to like solutions of pepsin in distilled water. In the latter series an equal volume of 0.5 per cent hydrochloric acid was added just before making the tests.

Formaldehyde and Pepsin.

Experiment V.—One gramme of pepsin was dissolved in 1000 cc. of water. 75 cc. of this solution were placed in flasks and additions made as follows:

Flask 1	received	3.75 cc.	formalin (20 per cent) =	formaldehyde	1:100
" 2	"	1.50	"	"	1:250
" 3	"	0.75	"	"	1:500
" 4	"	0.375	"	"	1:1000
" 5	"	0.15	"	"	1:2500
" C	"	no formalin and served as control.			

After standing at ordinary temperature for 24 hours, 15 cc. of each mixture were measured into a test-tube and an equal volume of 0.5 per cent hydrochloric acid and a few shreds of fibrin were added. The tubes were then placed in a water-bath which was kept at a temperature of 38°-40°. The fibrin in all of the tubes began swelling immediately and at the end of two or three minutes the liquid became cloudy, indicating that digestion was taking place. At the end of half an hour the fibrin in each tube was almost entirely dissolved, and 15 minutes later it disappeared completely. So far as could be observed there was no difference in the appearance of any of the tubes.

The original mixtures were set aside at ordinary room temperature and at the end of two weeks, and again at the end of four weeks, these were tested as outlined above. The results were exactly the same as in the first, *i. e.* the fibrin swelled immediately and disappeared completely in from one-half to three-quarters of an hour. The mixtures of pepsin-formaldehyde all remained clear and contained no deposits, with the exception of the control solution C. This soon began to give indications of decomposition; within three days the solution was cloudy and had a strong putrid odor; yet it was active, since after standing for a month it was able to digest fibrin as rapidly as when perfectly fresh.

In some other work carried on with solutions of pepsin in ordinary tap-water it was found that the ferment lost its activity within 24 hours and was no longer able to digest fibrin. On referring to the literature, however, it was found that certain inorganic salts, which are commonly found in tap-water, exert more or less effect on pepsin, some destroying it completely. The destruction of the ferment action in these cases is thus explained.

Formaldehyde and Pepsin-Hydrochloric Acid.

Experiment VI.—One gramme of pepsin was dissolved in 1000 cc. of 0.25 per cent hydrochloric acid. 75 cc. of this solution were placed in each of six flasks and additions of formaldehyde were made to these as in Experiment V. These mixtures of formaldehyde and ferment were made at the same time as those in the preceding experiment and the tests were made together.

15 cc. of each solution were measured into a test tube and fibrin added. The tubes were then placed in a water-bath and kept at a temperature of 38°-40°. Tests were made at the end of 24 hours, two weeks and four weeks. The results obtained were exactly the same as in the preceding experiment. In fact, if the tubes had not been labeled, it would have been impossible to distinguish one from another.

These results led us to doubt the statement of Loew that formaldehyde destroys the activity of pepsin. Consequently several sets of tests were made, in which the same amounts of ferment and of formaldehyde were used as were employed by him.

Experiment VII.—One gramme of ferment was dissolved in 10 cc. of water and 5 cc. of a 15 per cent solution of formaldehyde were added. In the first set the Kahlbaum pepsin was used, and in the second, a sample made by Parke, Davis & Co., labeled "Pepsin, Aseptic (Pepsin U. S. P. 1890) 1:3000." This was in the form of yellow scales, and was easily and completely soluble in water, and very active. The mixtures of ferment and formaldehyde were then allowed to stand in corked flasks for 24 hours. Inasmuch as formaldehyde in strong concentration rapidly hardens fibrin and renders it incapable of being digested, especially when allowed to act at an elevated temperature, the mixtures of ferment and formaldehyde were strongly diluted with 0.25 per cent hydrochloric acid just before making the test, and small shreds of fibrin were used. The dilutions made in each case were 1:15, 1:30, 1:60. Fibrin was added to portions of each, and the tubes then placed in a water-bath and kept at 40°. The fibrin in each tube was nearly dissolved in about a quarter of an hour and disappeared completely a few minutes later. At the end of five days, and again after three weeks, new portions of the ferment-formaldehyde solutions were diluted and tested as above. The results were exactly the same as those just given.

From this it would seem that Loew did not dilute his formaldehyde-ferment solution, and if so, his failure to obtain digestion would seem to be due, not to the action of formaldehyde on pepsin, but rather to the action of formaldehyde on the fibrin, altering it before the pepsin could act. This view is confirmed by the following experiments:

Experiment VIII.—Mixtures were made using the same amounts of both samples of pepsin, water, and formaldehyde. These were allowed to stand for 24 hours and were then tested as follows: 10 cc. of each mixture were diluted with an equal volume of 0.5 per cent hydrochloric acid, a few shreds of fresh fibrin were added, and the tubes were then kept at 40°. At the same time control tests were made, with small portions of the same mixtures diluted strongly with 0.25 per cent hydrochloric acid. At the end of half an hour, the fibrin in the tubes containing the very dilute mixture was nearly dissolved, whereas that in the other tubes

was dissolving slowly, although the amount of ferment present was much larger. The formaldehyde was evidently hardening the fibrin so rapidly that the pepsin could not accomplish its work completely. At the end of 3 hours there remained some shreds of fibrin still undissolved, and these gave no further evidence of dissolving during the following 6 hours, but appeared hardened.

The same tests were repeated at the end of three days, the mixtures being kept in the incubator for 24 hours in the meantime. The results obtained were exactly the same as in the preceding.

These results fully confirm the statement made by Simons, that formaldehyde has no apparent effect on peptic digestion. These results, taken in conjunction with those obtained in studying the action of formaldehyde on fibrin also confirm the statement made by Maybery and Goldsmith, that with an increase in the amount of formaldehyde there will be a decrease in the amount of fibrin digested in a given time. This, however, is due solely to the action of formaldehyde on fibrin and not to any alteration of the pepsin. In the experiments outlined above small portions of fibrin were used; if, however, larger amounts were employed some of the fibrin would be hardened by the formaldehyde before digestion could take place, and hence the process would be retarded. At the end of a given time there would still be undigested fibrin in the tubes in which formaldehyde was present.

Benedicenti found that proteids, albumin, fibrin, casein, etc., which had lost their power of being digested by gastric or pancreatic juices through the action of formaldehyde, recovered this power after being heated in a current of steam. The formaldehyde was removed from its combination with the proteid. Inasmuch as ammonia rapidly unites with formaldehyde, thus neutralizing its action, it seemed probable that ammonia would act in a manner similar to that of a current of steam, removing the formaldehyde from the proteid and thus restoring to the latter its property of being dissolved by ferments. Several experiments were made in order to determine whether ammonia would have this action on fibrin and on casein previously treated with formaldehyde.

Experiment IX.—Fresh fibrin was allowed to stand in a solution of formaldehyde 1:100 in a closed flask at ordinary temperature. At the end of 6, 24, and 48 hours portions were transferred to dilute ammonia, about 3.5 per cent in some cases, while in other cases very dilute ammonia was used. After allowing the ammonia to act for some time, the fibrin was washed and subjected to the action of pepsin-hydrochloric acid. At the same time portions of fresh fibrin and of formaldehyde-fibrin which had not been subjected to the action of ammonia were also added to pepsin-hydrochloric acid. Table IV shows the results.

TABLE IV.

Tube No.	Time of action of formaldehyde.	Time of action of ammonia.	Results.
1.	......	.....	Fibrin nearly dissolved in ½ hour; dissolved completely a few minutes later.
2.	6 hours.	......	Fibrin swollen slightly in 1 hour; liquid clear; nearly dissolved in 5 hours.
3.	"	3.5% NH_3 15 min. 40°.	Fibrin swollen slightly in 1 hour; liquid clear; partially dissolved in 5 hours.
4.	"	3.5% NH_3 30 min. 40°.	Fibrin swollen slightly in 1 hour; liquid clear; nearly dissolved in 5 hours.
5.	......	......	Fibrin nearly dissolved in 1 hour.
6.	24 hours.	......	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
7.	"	3.5% NH_3 5 hours.	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
8.	"	Trace NH_3 5 hours.	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
9.	......	......	Fibrin dissolved in about 1 hour.
10.	48 hours.	......	No change at the end of 6 hours.
11.	24 hours.	3.5% NH_3 24 hours.	Fibrin very slightly swollen; liquid clear at the end of 3 hours; partially dissolved in 6 hours.
12.	"	Trace NH_3 24 hours.	Fibrin very slightly swollen; liquid clear at the end of 3 hours; partially dissolved in 6 hours.

A test made with papaïn gave very similar results. Fibrin was allowed to stand for about 3 hours in formaldehyde 1:100 at 40°. It was then washed and placed in 3.5 per cent ammonia. After allowing the latter to act for 4 hours, the fibrin was washed and added to a strong solution of papaïn, and the tube kept in the incubator over night. On the following day there was no change in the appearance of the fibrin and the ferment solution was perfectly clear, thus showing that no digestion had taken place.

The results with casein agree with those obtained with fibrin. Formaldehyde was added to milk in the proportion of 1:100 and the mix-

ture kept at 40° for half an hour. At the end of that time a portion was removed and to this was added the calculated amount of a 3.5 per cent solution of ammonia necessary to neutralize the formaldehyde present. This mixture was likewise put at 40° for half an hour, then 10 cc. were removed and 1 cc. of rennet solution (1-1) added. No coagulation took place, even after several hours, but a precipitate of coarse floccules appeared.

These results indicate that ammonia will have but little if any effect on the proteid which has been altered by formaldehyde.

ACTION OF FORMALDEHYDE ON RENNET.

The rennet ferment used was the same as that employed in studying the action of formaldehyde on milk. Two series of experiments were made; one with dilute rennet solution (1-4), and the other with stronger rennet solution (1-1). The results were the same in both cases except that with the more dilute ferment solution coagulation took place more slowly. The conditions were the same as in the previous tests on milk, namely, 1 cc. of the ferment solution was added to 10 cc. of milk, and the tubes were then placed in a water-bath and kept at a temperature of 40°. In the absence of formaldehyde coagulation would occur in about three-quarters of an hour to an hour with the dilute ferment solution (1-4), and in about 10-15 minutes with a stronger solution (1-1). If, however, the milk was previously warmed to about 40° coagulation would result much more quickly, sometimes even in one or two minutes.

Formaldehyde and Dilute Rennet.

Experiment X.—Formalin was added in the following proportions to portions of 40 cc. of rennet solution (1-4) in flasks:

Flask	1	received	4 c.c. formalin (40 per cent) =	formaldehyde	....	1:25
"	2	"	2 "	"	....	1:50
"	3	"	1 "	"	....	1:100
"	4	"	0.5 "	"	....	1:200
"	5	"	0.2 "	"	....	1:500
"	6	"	0.1 "	"	....	1:1000
"	C	"	no formalin. This served as control.			

Tests were made after the mixtures had stood $1\frac{1}{2}$, 4, 7 and 14 days at ordinary room temperature. Table V shows the results obtained. In

the tests made on the 4th, 7th and 14th days the milk was previously warmed to 40° and then the rennet-formaldehyde mixture added.

TABLE V.

Tube No.	Formaldehyde.	Mixtures of Rennet and Formaldehyde tested at the end of 1½ days.	4 days	7 days.	14 days.
1.	1:25	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.
2.	1:50	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.
3.	1:100	Coagulation in about 2 hours.	Coagulation in about 2 hours.	Thick in 5 hours, solid later.	No coagulation even after 7 hours.
4.	1:200	Coagulation in about 1¼ hours.	Coagulation in about 1¼ hours.	Coagulation in about 1¼ hours.	Coagulation in about 2½ hours.
5.	1:500	Coagulation in about 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1¼ hours.
6.	1:1000	Coagulation in about 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1 hour.
C.	......	Coagulation in 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1 hour.

It is evident from this experiment that solutions of rennet containing formaldehyde in strengths of 1:25 and 1:50 will apparently completely lose their ferment action in less than 36 hours. Mixtures containing formaldehyde 1:100 apparently slowly lose their power of coagulating milk, and hence retard the coagulation which, however, still takes place even after an exposure of 14 days. Mixtures containing formaldehyde 1:200 or less practically seem to have no effect on rennet. This loss of ferment action is not, however, due to destruction of rennet but rather, as will be shown later, to the action of formaldehyde on the casein of the milk, rendering this non-coagulable. This action corresponds to that of formaldehyde on egg albumen, on blood serum and on fibrin.

The apparent action of formaldehyde on rennet is well shown in the preceding experiment. This is because the weak rennet solution acts slowly on the milk and hence the formaldehyde has time to act on the casein. When a stronger rennet solution is used, as in Experiment XI, the formaldehyde is not given the time to alter the casein. Hence in Experiment XI coagulation takes place, or is but slightly retarded as compared with Experiment X.

Formaldehyde and Strong Rennet.

Experiment XI.—Formalin was added to portions of 20 cc. of the rennet solution (1-1), in flasks, in the following proportions:

Flask 1	received	1 cc. of formalin (40 per cent)=formaldehyde		1:50
" 2	"	0.5	" " "	1:100
" 3	"	0.25	" " "	1:200
" 4	"	0.1	" " "	1:500
" C	"	no formalin. Served as control.		

These mixtures were allowed to stand at ordinary room temperature, and were tested at intervals during a period of 5 weeks. The results are given in Table VI.

TABLE VI.

Tube No.	Formaldehyde.	Mixtures of Rennet and Formaldehyde tested at the end of 1 hour.	24 hours.	4 days.	11 days.	35 days.
1.	1:50	Coagulation in about 1¼ hrs.	Thick in 30 min.; solid in 40 min.	Thick in 1 hr.; solid later.	....	....
2.	1:100	......	Coagulation in 10 min.	Thick in 10 min.; solid in 15 min.	Coagulation in about 30 min.	Coagulation in 15 min.
3.	1:200	......	Coagulation in 10 min.	Thick in 5 min.; solid in 10 min.	Coagulation in about 30 min.	Coagulation in 10 min.
4.	1:500	......	Coagulation in 7 min.	Coagulation in 5 min.	Coagulation in 20 min.	Coagulation in 7 min.
C.	......	Coagulation in 10 min.	Coagulation in 3 min.	Coagulation in 5 min.	Coagulation in 15 min.	Coagulation in 7 min.

From these results it will be seen that formaldehyde, when added to a strong solution of rennet ferment, even in the proportion of 1:50, exerts no apparent effect on the ferment. The mixtures of ferment and formaldehyde were as active at the end of five weeks as when fresh. The slight differences in time required for coagulation by the same solution of ferment on different days are most probably due to differences in the composition or in the temperature of the milk. Market milk was used, and is liable to vary slightly from time to time.

From the tables it will be seen that the ferment solutions which contained the most formaldehyde did not produce a coagulation as rapidly as those in which formaldehyde was present in smaller amount or was entirely absent. This is no doubt due to the rapid alteration which the casein undergoes, especially at a temperature of about 40°, rather than to a change in the ferment. The following tests favor this view.

Experiment XII.—0.25 cc. of 40 per cent formalin was added to 100 cc. of fresh milk, which was already warmed to 40°, making the ratio of formaldehyde 1:1000. This was kept at 40° and portions of 10 cc. were removed and tested at intervals with 1 cc. of rennet solution (1-1).

After an exposure to formaldehyde of 15 minutes the milk would coagulate in 10 to 15 minutes. After standing for an hour, it would thicken in about 25 minutes, but would not become solid till some time later. When tested at the end of two hours the same results were obtained.

On comparison with Experiments III and IV, it will be noticed that when formaldehyde is added to milk in the ratio of 1:1000 there is slow action at ordinary room temperature. After standing for a day or so, rennet will give a coagulation almost as promptly as with fresh milk. If, however, the formaldehyde is added to milk and the mixture kept at about 40°, the action is more rapid, as shown above.

In order to render perfectly clear the action of formaldehyde in Experiments X and XI, attention should be called to the fact that the formaldehyde rennet mixtures were added in portions of 1 cc. to 10 cc. of milk. Consequently the resultant mixture of milk, rennet and ferment contained approximately one-tenth the amount of formaldehyde in the original rennet mixture. Thus, the rennet-formaldehyde mixture (1:50) on addition to milk yields a solution containing formaldehyde 1:500. Now, on reference to Experiments III and IV, it will be seen that the addition of formaldehyde (1:500) to milk altered the casein in 4½ hours and 1 hour respectively to such an extent that in the former case (dilute rennet) no coagulation resulted, whereas in the latter case (strong rennet) coagulation was retarded. It required 1¼ hours to coagulate, whereas in the control test the milk coagulated in 15 minutes. This, it will be seen, corresponds exactly to the behavior of formaldehyde-rennet mixture 1:50 in Experiments X and XI.

If, however, formaldehyde is added to warm milk the action is much more rapid. Portions of 10 cc. each of milk were warmed to 40° and 0.25 cc. of formalin (40 per cent) were then added to each, making the proportion 1:100. After these mixtures had stood for 1, 3, 10 and 15 minutes, 1 cc. of the rennet solution (1-1) was added. Coagulation resulted within 1 or 2 minutes in the first and second tubes, and in about 5 minutes in the third. The milk in the fourth tube did not give any indication of coagulating during the first quarter of an hour. Then it began to thicken, gradually became thicker and finally became almost solid in about an hour.

ACTION OF FORMALDEHYDE ON PAPAÏN.

The papaïn used was made by Kahlbaum. It was a very light gray powder, readily soluble in water, forming a clear solution. It was not, however, nearly as active, weight for weight, as the pepsin, and consequently was used in more concentrated solution.

Experiment XIII.—5 grammes of the ferment were dissolved in 100 cc. of distilled water. This solution was divided into portions of 75 cc. each, which were placed in flasks and formaldehyde was added as follows:

Flask	1	received	3.75 cc. of formalin (20 per cent) =	formaldehyde		1:100
"	2	"	1.50	"	"	1:250
"	3	"	0.75	"	"	1:500
"	4	"	0.375	"	"	1:1000
"	C	"	no formalin and served as control.			

After standing for 24 hours at ordinary room temperature, 10 cc. of each mixture were placed in test-tubes; an equal volume of a 0.4 per cent solution of hydrochloric acid and a few small shreds of fibrin were added to each tube. The tubes were then placed in a water-bath and kept at a temperature of 38°-40° for several hours.

Within a few minutes the fibrin in all of the tubes had become swollen, owing to the action of the acid. There was, however, no sign of digestion in any of the tubes in which formaldehyde was present. Even after being kept at that temperature for 24 hours the liquid was perfectly clear and there was no diminution in the amount of the fibrin. Digestion did proceed, however, in tube C, which served as a control. At the end of two hours the liquid was cloudy and about half of the fibrin had dissolved; two or three hours later the fibrin had entirely disappeared.

It did not seem probable that the failure of papaïn to digest fibrin in the above experiment could be due to any action of the formaldehyde on the fibrin. Fibrin, as seen in Experiment II, which has been allowed to stand for three days in a formaldehyde solution of the same strength as that used in mixtures 3 and 4 is quite readily digested by pepsin and by pancreatin. It was found, however, that fibrin which had been exposed to the action of a formaldehyde solution 1:1000 for half an hour at 40° will be digested by papaïn. Moreover, a solution of papaïn in hydrochloric acid, containing formaldehyde in the proportion of 1:1000, will not digest fresh fibrin.

The following experiments will serve to explain the previous results:

Experiment XIV.—1. Some fresh fibrin was added to a solution of formaldehyde 1:1000 and kept at 40°. At the end of 3 and again at the end of 7 hours small portions were taken out, thoroughly squeezed in order to remove the formaldehyde adhering, and then tested with the control solution C used above. Even after standing over night at a temperature of 37°-40° they showed no signs of digesting. The liquid was perfectly clear and there was no diminution in the amount of the fibrin, which, however, had become swollen.

2. Another test was made in order to determine whether fibrin would be altered in less time than three hours so that it would become non-digestible.

5 grammes of papaïn were dissolved in 500 cc. of 0.15 per cent hydrochloric acid, thus making a ferment solution of nearly double the strength of the one used in the preceding experiment. Fibrin was added to a solution of formaldehyde 1:1000 and kept at 40°. At intervals of one-half hour small portions were removed, washed, and added to portions of 15 cc. of the ferment solution, and the tubes kept at 40°. Three tests were made, in which the fibrin had been exposed to the action of the formaldehyde for one-half, one and one and one-half hours, respectively. In none of these did digestion take place. Even after standing in the incubator for 24 hours the fibrin was unchanged in appearance and the liquid was perfectly clear in each tube. In the control tube, however, the liquid became cloudy in a short time, and the fibrin was almost entirely digested within three hours.

3. 1 gramme of papaïn was dissolved in 50 cc. of water and the solution divided into three portions, *a*, *b* and *c;* to *b* formaldehyde was added in the proportion of 1:1000. The solutions were then set aside at ordinary room temperature for 24 hours. Fresh fibrin was then added to *a* and *b*, whereas *c* received fibrin which had stood for 24 hours in formaldehyde 1:1000. An equal volume of 0.3 per cent hydrochloric acid was added to each and the tubes were then placed in the water-bath at 40°. At the end of an hour the liquid in tube *a* was cloudy; the fibrin slowly dissolved during the afternoon. The fibrin in tubes *b* and *c* gave no indications whatever of digesting after being kept at that temperature for several hours. The fibrin was swollen, but there was no diminution in its amount and the liquid was perfectly clear.

4. 5 grammes of papaïn were dissolved in 250 cc. of water and formaldehyde was added in the proportion of 1:1000. The solution was then allowed to stand for 24 hours at ordinary temperature, then divided into two portions. Ammonia was added to the first in sufficient amount to

exactly neutralize the formaldehyde, while double this quantity of ammonia was added to the second. After standing for three hours at ordinary temperature portions of each of these mixtures were placed in tubes, the free ammonia neutralized with dilute hydrochloric acid, and then an equal volume of 0.3 per cent hydrochloric acid and fibrin were added. The tubes were then placed in the incubator at 38°-40° and examined on the following day. The fibrin in both tubes was swollen, but none had dissolved; both solutions were perfectly clear.

The same experiment was repeated after the ammonia had been allowed to act for eight hours. The results were exactly the same as in the preceding.

It was found, however, that ammonia even in very small amount destroyed the ferment. A few drops of a dilute solution of ammonia added to the above solution of papaïn and allowed to act for half an hour rendered it incapable of digesting fibrin.

It is evident from the above results that formaldehyde interferes with papaïn digestion, both by destroying the ferment as well as by hardening the fibrin.

ACTION OF FORMALDEHYDE ON COMMERCIAL PANCREATIN.

Commercial pancreatin (Parke, Davis & Co.) was employed in the following experiments:

Experiment XV.—5 grammes of the yellow powder were dissolved in 400 cc. of water and formaldehyde was added in the same proportions as in the case of papaïn. Experiment XIII, namely, 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. 10 cc. of the control solution, to which an equal volume of one or two per cent Na_2CO_3 solution was added would dissolve a few shreds of fibrin in two or three hours. The proteolytic action was therefore quite strong, though not as marked as that of the aqueous extract of pancreatic gland employed in experiments presently to be described. Inasmuch as this solution possessed very weak diastatic power its action on starch paste was not tried.

After the solutions of ferment and formaldehyde had been made, they were allowed to stand at ordinary temperature for 24 hours. Then 10 cc. were taken from each mixture, diluted with an equal volume of one per cent Na_2CO_3 solution, and small shreds of fibrin added. The tubes

were then placed at a temperature of 38°-40°, and examined occasionally during the next 24 hours. The results are given in Table VII.

TABLE VII.

PANCREATIN-FORMALDEHYDE MIXTURE (24 HOURS OLD), TESTED WITH FIBRIN.

Tube No.	Formaldehyde.	Digested at 38°–40° for 2 hours.	4 hours.	6 hours.	24 hours.
1..	1:100	No change, except that the fibrin seems hardened.	Same as before.	Same as before.	Same as before.
2..	1:250	No change, except that the fibrin seems hardened.	"	"	"
3..	1:500	No change.	"	"	Same as before; fibrin slightly hardened.
4..	1:1000	No change.	"	"	Same as before; fibrin slightly hardened.
C..	....	Fibrin nearly dissolved.	Fibrin entirely dissolved.	"	

There was no diminution in the amount of the fibrin in the first four tubes and no indication whatever that digestion was taking place. It appears from these results that formaldehyde, even in very small amount, prevents pancreatic digestion of proteid material, and that this is due to alteration of the ferment rather than to a change in the material acted upon. It has already been stated under Experiment II that fibrin, when exposed to the action of dilute formaldehyde solution (1:1000) for 24 hours is digested, though the process is retarded. This fact is brought out clearly in Test 3 below.

The following experiment was made in order to demonstrate positively the action of formaldehyde on trypsin. The solution of pancreatin used was the same as that employed in the preceding experiment.

Experiment XVI.—Tube 1 contained pancreatin solution and fibrin, and served as a control.

Tube 2 contained pancreatin and formaldehyde 1:1000 24 hours old, and fibrin.

Tube 3 contained pancreatin to which was added fibrin which had stood in formaldehyde 1:1000 for 24 hours at room temperature.

The results are given in Table VIII.

TABLE VIII.

Tube No.	Digesting mixtures kept at 38°–40°, and examined at the end of 2 hours.	3 hours.	6 hours.
1..	Fibrin dissolving.	Fibrin dissolved.	
2..	No change.	No change.	No change; fibrin is slightly hardened.
3..	"	Fibrin digesting slowly.	Fibrin dissolved.

It is evident therefore that fibrin which had been exposed to the action of a very dilute formaldehyde solution is soon rendered very resistant to the action of commercial pancreatin. The latter, however, is quickly altered and rendered incapable of digesting fibrin.

ACTION OF FORMALDEHYDE ON AN EXTRACT OF PANCREATIC GLAND.

The results with commercial pancreatin were confirmed with an aqueous extract of the fresh pancreatic glands. This extract was also used for the purpose of studying the effect of formaldehyde on the diastatic ferment.

Experiment XVII.—Two fresh glands were cut up as finely as possible and a litre of water was added. The mixture was allowed to stand over night in a cool place. It was then filtered and the clear filtrate was divided into portions, to which formaldehyde was added in the same proportions as in the case of the pancreatin solutions, namely, 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. These mixtures were allowed to stand at ordinary room temperature for 24 hours. Then 10 cc. of each mixture were diluted with an equal volume of two per cent sodium carbonate, a few shreds of fibrin added, and the tubes placed in the water-bath at 38°-40°. The results were as follows (Table IX):

TABLE IX.

PANCREATIC EXTRACT AND FORMALDEHYDE MIXTURES, 24 HOURS OLD

Tube No.	Formaldehyde.	Digested at 38°–40° for 2 hours.	4 hours.	6 hours.	24 hours.
1..	1:100	No change, except that the fibrin seems hardened.	Same as before.	Same as before.	Same as before.
2..	1:250	No change, except that the fibrin seems hardened.	"	"	"
3..	1:500	No change.	"	"	"
4..	1:1000	Fibrin dissolving slowly.	Fibrin about half dissolved.	Fibrin almost entirely dissolved.	
C..	....	Fibrin nearly dissolved.	Fibrin dissolved.		

Only one set of tests was made with these mixtures, since it was found that when formaldehyde had been added to the solution in the proportion of 1:500 or stronger and allowed to act for 24 hours, digestion of fibrin would not take place: and when added in smaller amount, 1:1000, the activity of the ferment was considerably lessened.

Experiment XVIII.—A stronger solution of the trypsin ferment was made than that used in the preceding experiment. Two beef glands, finely divided, were suspended in 500 cc. of water. The mixture was allowed to stand 24 hours and was then filtered. Formaldehyde was added to portions of the clear filtrate in the ratio of 1:500 and 1:1000, while another portion received none and served as a control solution. The solutions were allowed to stand for 24 hours longer. Then 10 cc. of each mixture were diluted with 10 cc. of a two per cent sodium carbonate solution and tested with fresh fibrin, and also with fibrin that had been exposed to the action of formaldehyde 1:500 and 1:1000 for 24 hours. The results are given in Table X.

TABLE X.

MIXTURES OF TRYPSIN AND FIBRIN-FORMALDEHYDE DIGESTED AT 38°–40°.

24 hours' Mixture of trypsin-formaldehyde.	Material used.	Examined at the end of	
		$\frac{1}{2}$ hour.	$1\frac{1}{2}$ hours.
Formaldehyde, 1:500.	Fibrin.	No change.	Fibrin nearly dissolved.
	Fibrin-formaldehyde, 1:500.	No change.	Fibrin dissolving slowly.
	Fibrin-formaldehyde, 1:1000.	No change.	Fibrin dissolved.
Formaldehyde, 1:1000.	Fibrin.	Fibrin dissolving slowly.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:500.	No change.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:1000.	Fibrin nearly dissolved.	Fibrin dissolved.
Plain trypsin.	Fibrin.	Fibrin nearly dissolved.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:500.	Fibrin dissolving slowly.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:1000.	Fibrin nearly dissolved.	Fibrin dissolved.

At the end of six hours the solutions in the last three tubes had a strong, putrid odor, whereas none of the others possessed any such odor.

When formaldehyde is added in rather strong concentration (1:100 or stronger) to an aqueous extract of the pancreatic gland, rich in ferment and proteid matter, a coarse, voluminous precipitate is thrown down. It was thought that possibly the ferment might be enclosed within this precipitate and prevented from exerting its action through mechanical

interference, although it might still possess its proteolytic property. This was not found to be the case, however, as will be seen from the following experiment.

Experiment XIX.—Two fresh glands were crushed in a mortar with fragments of glass, 250 cc. of water were added, and the mixture allowed to stand over night. It was then filtered, and formaldehyde was added to portions of the clear filtrate in the proportion of 1:25, 1:100, 1:500 and 1:1000; another portion was reserved for control tests. These mixtures were again allowed to stand over night. At the end of that time the first two flasks contained a coarse precipitate, while the other three mixtures were almost perfectly clear. Portions from the first and second flasks were ground finely in a mortar with fragments of glass, an equal volume of one per cent sodium carbonate added, and then small portions of fibrin. The tubes were then placed in an incubator and kept at a temperature of 35°-40° over night. At the end of 24 hours the fibrin in these two tubes gave no indication whatever of dissolving: on the contrary, it appeared hardened. The fibrin in the control tube, however, was completely dissolved within a few hours and the solution had the strong, putrid odor characteristic of tryptic digestion.

Inasmuch as fibrin is rapidly hardened by formaldehyde, especially when the latter is allowed to act at a temperature of 35°-40°, it seemed possible that failure to undergo digestion by a solution of trypsin containing formaldehyde might be made due to this cause. Consequently portions of each of the mixtures of ferment mentioned above were diluted with 20 parts of 0.5 per cent sodium carbonate solution, fibrin was added, and the tubes were placed in the incubator over night. At the end of 24 hours the fibrin in the first tube was slightly hardened, but had not dissolved. The fibrin in the second, third, and fourth tubes had apparently undergone no change whatever; that in the fifth, or control, tube was dissolving slowly. A few hours later the fibrin in the fourth tube was nearly dissolved, and that in the control was completely dissolved.

The following experiment was made in order to determine whether neutralization of the formaldehyde with ammonia would restore the proteolytic property to the ferment.

Experiment XX.—An extract was made as in the above experiments, using two glands and 500 cc. of water. Formaldehyde was added in the proportions of 1:25, 1:100, 1:1000, while another portion was reserved for control experiments. After allowing these mixtures to stand over night, the calculated amounts of ammonia required to neutralize the

formaldehyde were added to portions of each of the mixtures, and they were then allowed to stand for five hours. An equal volume of one per cent sodium carbonate solution and a few shreds of fibrin were added, and the tubes were then placed in the incubator over night. Next morning the fibrin in the first and second tubes had undergone no change; that in the third tube had shown no indications of dissolving during the first seven or eight hours, but later it did dissolve completely. The fibrin in the control tube dissolved completely within about three hours.

A few drops of ammonia were added to another portion of the control solution of ferment, allowed to act for five hours, and then the solution was tested as above. The fibrin in this tube was dissolved with about the same rapidity as that in the control test, showing that a small amount of free ammonia has but very little if any action on trypsin.

The conclusions that are to be drawn from these experiments are that formaldehyde exerts a powerful action on trypsin. When present even in so small an amount as 1:1000, digestion of fibrin is greatly retarded. When added to a strong extract of the ferment in the proportion of 1:500, digestion of fibrin will take place but very slowly. If, however, formaldehyde is added in the same ratio to a weaker solution of the ferment, digestion will fail to take place. That formaldehyde alters the trypsin and renders it inactive is seen from the results in Experiment XIX; for if failure to digest fibrin were due to the hardening action of formaldehyde alone, dilution of the ferment-formaldehyde mixture should make the proportion of formaldehyde so small that its action would be imperceptible, in which case the ferment should dissolve the fibrin. The addition of ammonia will not restore to the ferment its proteolytic property.

ACTION OF FORMALDEHYDE ON AMYLOPSIN.

The influence of formaldehyde on the diastatic ferment of the pancreatic gland was tested in connection with the experiments on trypsin just described. In the first few tests, a starch paste made from corn starch was employed; this was not suited for experiments with diastatic ferments, however, since corn starch is not so easily and rapidly digested as potato starch. Consequently the latter was used instead in the later tests, and only these tests will be given.

Experiment XXI.—The mixtures of extract and formaldehyde employed were those described in Experiment XIX. After these mixtures had stood for about 24 hours, 10 cc. of each were diluted with 10 cc. of a one per cent sodium carbonate solution, and 20 cc. of a freshly prepared one per cent starch paste were added. Two other tubes were prepared, using the formalin-ferment mixtures Nos. 1 and 2 (containing formaldehyde 1:25 and 1:100 respectively), which had previously been ground up finely with fragments of glass. The tubes were kept in the incubator and small portions of each were tested from time to time with iodine solution. The results are given in Table XI.

TABLE XI.

PANCREATIC SUSPENSION AND FORMALDEHYDE (KEPT FOR 24 HOURS AT ORDINARY TEMPERATURE), THEN STARCH ADDED AND RESULTANT MIXTURE KEPT AT 38°–40° AND TESTED WITH IODINE SOLUTION.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 24 hours.
1. (ground.)	1:25	" " " "
2.	1:100	Violet in 15 min.; color gradually became lighter, pink at the end of 24 hours.
2. (ground.)	1:100	Deep blue at the end of 24 hours.
3.	1:500	Pink in 15 min.; colorless in 30 min.
4.	1:1000	Colorless in 15 min. or less.
C.	....	" " "

Experiment XXII.—The mixtures of ferment and formaldehyde were those described in Experiment XX. The tests were made in the same way as in the preceding experiment, and the results are given in Table XII.

TABLE XII.

MIXTURES OF FERMENT-FORMALDEHYDE (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 2 hours; deep violet in 3 hours.
2.	1:100	Violet at the end of 15 min.; colorless at the end 30 min.
3.	1:1000	Colorless in 15 min., or less.
C.	......	" " "

Experiment XXIII.—Portions of the same mixtures as those used in Experiment XXII were treated with the calculated amounts of ammonia necessary to neutralize the formaldehyde present, allowed to stand at ordinary temperature for five hours, and then tested in the same manner as above. A few drops of ammonia were also added to a portion of the control mixture at the same time. The results were quite similar

to those given in Experiment XXII. although the conversion was slower in each case.

TABLE XIII.

MIXTURES OF FERMENT-FORMALDEHYDE-AMMONIA (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 24 hours.
2.	1:100	Deep blue at the end of one hour; color gradually became lighter; pink at the end of 24 hours.
3.	1:1000	Colorless in about 1 hour.
C.	......	Colorless in about 30 minutes.

These results show that ammonia will not remove the formaldehyde that is held in combination by the ferment, and thus restore to the latter its amylolytic property. On the contrary, the presence of free ammonia seems to hinder the action of the ferment. This view is confirmed by the following test, made with mixture No. 3 (containing formaldehyde 1:1000).

To two portions of 10 cc. each of this solution 1 and 4 cc. respectively of a 3.5 per cent solution of ammonia were added, and the mixtures allowed to stand for about three hours. An equal volume of starch paste was then added to each and the tubes were placed in the incubator over night. Next morning small portions of each were tested with iodine, after neutralizing the free ammonia with dilute acetic acid. In one case a deep wine color was produced, while in another, in which ammonia had been added in larger amount, a deep violet color was produced. A control test made with the same mixture of ferment and formaldehyde gave no color with iodine after 15 minutes at 40°.

From the above results it will be seen that formaldehyde has a depressing action on the diastatic ferment of the pancreas, though this action is not so marked as in the case of trypsin. Formaldehyde added to an active solution of trypsin in the proportion of 1:500 will completely destroy the action of the ferment, unless the solution is an exceedingly active one. Even when present in so small an amount as 1:1000 the action of the ferment is greatly retarded. On the other hand, formaldehyde added to a solution of amylopsin in the proportion of 1:500 exerts but little action on the ferment after being allowed to act for 24 hours. When present in the ratio of 1:100 conversion of starch is greatly retarded, but will still take place.

Like trypsin, amylopsin seems to be destroyed, and not rendered inactive merely by mechanical interference; and like trypsin, papaïn and saliva, its properties are not restored by adding the calculated amount of ammonia required to neutralize the formaldehyde.

ACTION OF FORMALDEHYDE ON PTYALIN.

Several samples of ptyalin were tested, but inasmuch as these possessed little or no action, saliva was employed instead. The experiments were carried on in general in the same manner as those with amylopsin, and the results were very similar to those obtained with that ferment; they agree also with the results obtained with malt diastase to be described.

When formaldehyde was added to saliva in small amount it had very little effect on ptyalin; if added in larger amount, and the mixture kept at ordinary temperature, it had a depressing action, but did not completely destroy the ferment for several days. If such a mixture were kept at about 40° for some time, however, the action of the formaldehyde was more marked, and the ferment was eventually destroyed.

Experiment XXIV.—Fresh saliva was collected, filtered, and divided into portions of 20 cc. each. Formaldehyde was added to these in the proportions of 1:100, 1:250 and 1:1000; another portion was reserved for control tests. These mixtures were allowed to stand in corked flasks for 24 hours at ordinary temperature. At the end of that time 1 cc. of each was added to 25 cc. of freshly prepared one per cent potato starch paste, the tubes placed in the water-bath at 40°, and at intervals portions from each tube were tested with iodine solution. The results are given in Table XIV.

TABLE XIV.

MIXTURES OF SALIVA-FORMALDEHYDE (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Color gradually changed to light wine-red at the end of 5 hours; colorless next morning.
2.	1:250	Light wine-red at the end of ½ hour; colorless in ¾ hour.
3.	1:1000	Nearly colorless in 10 min.; colorless in less than 20 min.
C.	......	Colorless in less than 5 min.

The tests were repeated on the following day with almost exactly the same results. Conversion, however, took place slightly more slowly in tubes Nos. 1, 2 and 3. At the same time a series of tests was made in like manner, using portions of these same saliva-formaldehyde mixtures which had been kept in the incubator at about 40° for 24 hours. Conversion took place much more slowly in these, showing that the formalin exerts more effect on the ferment if allowed to act on it for some time at an elevated temperature. The results are given in Table XV.

TABLE XV.

MIXTURES OF SALIVA-FORMALDEHYDE (24 HOURS AT 40°) AND STARCH TESTED WITH IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Deep wine-red at end of 7 hours.
2.	1:250	Light " " "
3.	1:1000	Colorless in about 1½ hours.
C.	......	Colorless in less than 5 min.

These results were confirmed in the following experiment:

Experiment XXV.—Saliva-formaldehyde mixtures were made in the same manner as in the preceding experiment. Each was divided into two portions, one of these being kept at ordinary temperature, and the other at 35°-40° during the time the experiment was carried on. Tests were made at the end of 4 hours, 24 hours, 3 days and 9 days. 25 cc. of freshly prepared starch paste and 1 cc. of the saliva-formaldehyde mixture were used in each case. The results are given in Tables XVI and XVII; the former table is with the mixtures that had been allowed to stand at ordinary temperature, the latter with those kept at 35°-40°.

TABLE XVI.

Tube No.	Formaldehyde.	Mixtures of Saliva–Formaldehyde (ordinary temperature) and Starch tested with Iodine at the end of			
		4 hours.	24 hours.	3 days.	9 days.
1.	1:100	Colorless in about ½ hour.	Colorless in about 6 hours.	Light violet at the end of 20 hours.	Deep violet at the end of 24 hours.
2.	1:250	Colorless in about 20 minutes.	Colorless in about ¾ hour.	Nearly colorless at the end of 10 hours.	Light wine-red at the end of 24 hours.
3.	1:1000	Colorless in less than 10 minutes.	Colorless in about 15 minutes.	Colorless in about 4 hours.	Colorless in about 12 hours.
C.	......	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.

TABLE XVII.

Mixtures of Saliva–Formaldehyde (kept at 35°–40°) and Starch tested with Iodine at the end of

Tube No.	Formaldehyde.	4 hours.	24 hours.	3 days.	9 days.
1.	1:100	Wine-red at the end of 2 hours. Colorless later.	Deep violet at the end of 20 hours.	Deep blue at the end of 20 hours.	Deep blue at the end of 24 hours.
2.	1:250	Colorless in about ¾ hour.	Nearly colorless at the end of 20 hours.	Violet at the end of 20 hours.	Deep blue at the end of 24 hours.
3.	1:1000	Colorless in less than 10 minutes.	Nearly colorless at the end of 1 hour.	Wine-red at the end of 10 hours; colorless next morning.	Deep violet at the end of 24 hours.
C.	......	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.

Experiment XXVI.—A third short series was made in the following manner: Formaldehyde was added to each of two portions of 20 cc. of saliva in the proportions of 1:100; one of these mixtures was placed in a corked test-tube and kept in the incubator for five days at a temperature varying from 30°-40°, while the other was kept at ordinary room temperature. At the end of five days tests were made as in the preceding experiments. The saliva-formaldehyde mixture that had stood at ordinary temperature gave slow conversion; when tested at the end of ten hours with iodine solution a slight red color was given. The other mixture gave no conversion at all: at the end of 24 hours a deep blue color was produced with iodine. A control test made at the same time with some of the same saliva gave conversion in less than ten minutes.

Attempts were made to remove the formaldehyde from its combination with the ferment by means of ammonia. As in the case of papaïn, trypsin, and amylopsin, these attempts were unsuccessful. Ammonia was added to a portion of a saliva-formaldehyde mixture (formaldehyde 1:500) several days old and the tube was set aside for three hours. The free ammonia was then carefully neutralized with very dilute acetic acid, and the saliva was then added to starch paste. A control test was also made with the same mixture of saliva and formaldehyde. Conversion took place slowly in both, but with the same rapidity; when tested with iodine solution the same colors were produced by both mixtures.

That the formaldehyde acts on the ferment and not on the starch is proven by the following test: Formaldehyde was added to a fresh one per cent starch paste in the proportion of 1:100, the tube corked, and

kept at 35°–40° for five days. Saliva was then added, as in the preceding tests. Complete conversion resulted in less than ten minutes.

It is evident from these experiments that formaldehyde in very small amount exerts but very little action on ptyalin, unless the mixture is allowed to stand for several days or is kept at a temperature slightly above ordinary room temperature for a few hours. When present in larger amount the action is more marked. The ferment is slowly destroyed at ordinary temperature, though it does not completely lose its power even when exposed to the formaldehyde for several days. If, however, the formaldehyde-saliva mixture is kept at an elevated temperature the ferment is destroyed much more rapidly.

ACTION OF FORMALDEHYDE ON MALT DIASTASE.

A water extract of ground malt was used, and also malt itself. The results were exactly the same in both cases. In Experiments XXVII, XXVIII and XXIX starch paste made from corn starch was used, whereas in the remaining experiments potato starch was used.

Experiment XXVII.—100 grammes of ground malt were added to 1000 cc. of water and the mixture allowed to stand for about three hours with occasional shaking. It was then filtered clear, divided into portions, and formaldehyde added in the proportions of 1:100, 1:250, 1:1000 and 1:2000; another portion was reserved for control tests. These solutions were then tested after standing for two and for eight days. 5 cc. of each mixture were added to 25 cc. of fresh one per cent starch paste (corn), and the mixtures were kept at 58°-60°. From time to time small portions of each mixture were tested with iodine solution. The results are given in Table XVIII.

TABLE XVIII.

Tube No.	Formaldehyde.	Mixtures of Malt Extract and Formaldehyde Tested with Starch and Iodine after standing for 2 days.	8 days.
1.	1:100	Light violet at the end of 2 hours.	Light violet at the end of 4 hours.
2.	1:250	Colorless in about ¾ hour.	Light violet in 30 min.; colorless in about ¾ hour.
3.	1:1000	" " "	Pink in 30 min.; colorless in about ¾ hour.
4.	1:2000	" " "	Pink in 30 min.; colorless in about ¾ hour.
C.	......	Blue at the end of 2 hours.	Blue at the end of 4 hours.

A duplicate set of solutions made in the same way and at the same time gave exactly the same results.

Experiment XXVIII.—Another set of solutions was made in the same manner as above and formaldehyde was added in the proportions of 1:100, 1:500 and 1:1000. These were tested with two per cent and with one per cent starch paste (corn) after standing for 24 hours. In this series 1 cc. of the ferment solution was added to 20 cc. of the starch.

TABLE XIX.

Mixtures of Malt Extract and Formaldehyde Tested with Starch Paste and Iodine, after Standing 24 Hours.

Tube No.	Formaldehyde.	2 per cent starch.	1 per cent starch.
1.	1:100	Deep violet at the end of 4 hours.	Light violet at the end of 3 hours.
2.	1:500	Light violet at the end of 4 hours.	Colorless in 1 hour.
3.	1:1000	Colorless in about 3 hours.	Colorless in 1 hour.
C.	......	Nearly colorless in 5 hours.	Light violet at the end of 2 hours.

Experiment XXIX.—Ten grammes of malt and 100 cc. of water were placed in each of half a dozen flasks, and formaldehyde was added in the proportions of 1:100, 1:250, 1:500, 1:750 and 1:1000; the sixth mixture was reserved for control tests. These mixtures were tested at the end of 3, 9 and 20 days, using 5 cc. of the clear solution and 25 cc. of fresh one per cent starch paste. The results are given in Table XX.

TABLE XX.

		Mixtures of Malt Extract and Formaldehyde tested with Starch and Iodine at the end of		
Tube No.	Formaldehyde.	3 days.	9 days.	20 days.
1.	1:100	Bluish violet at the end of 2 hours.	Bluish violet at the end of 3 hours.	
2.	1:250	Light violet at the end of 2 hours.	Colorless at the end of 3 hours.	All the same as when tested on the 9th day.
3.	1:500	Nearly colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
4.	1:750	Colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
5.	1:1000	Colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
C.	......	Deep blue at the end of 2 hours.	Deep blue at the end of 2 hours.	

Experiment XXX.—An extract of malt diastase was prepared as described in Experiment XXVII. Formaldehyde was added to portions

of this in the proportions of 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. The mixtures were allowed to stand at ordinary temperature for 24 hours. 5 cc. of each were then added to 25 cc. of a freshly prepared one per cent starch paste made from potato starch and the tubes were kept at 60°. When tested with iodine solution at the end of fifteen minutes, all showed complete conversion. These results did not seem to agree with those previously obtained. It was found, however, that formaldehyde in rather strong concentration will destroy malt diastase when allowed to act on the latter for a few hours at 60°. This, taken in conjunction with the fact that corn starch is not so easily digested as potato starch, will explain the apparent differences in the results.

On the following day the above tests were repeated, and at the same time another series was made with portions of the same mixtures of extract and formaldehyde which had been heated to 60° for four hours. The results are given in Tables XXI and XXII.

TABLE XXI.

MIXTURES OF MALT EXTRACT AND FORMALDEHYDE TWO DAYS AT ORDINARY TEMPERATURE), TESTED WITH STARCH AND IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Colorless in about 20 minutes.
2.	1:250	Colorless in less than 15 minutes.
3.	1:500	" " "
4.	1:1000	" " "
C.	......	Deep red at the end of 2 hours.

TABLE XXII.

MIXTURES OF MALT EXTRACT AND FORMALDEHYDE (KEPT AT 60° FOR FOUR HOURS), TESTED WITH STARCH AND IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Deep violet at the end of 5 hours.
2.	1:250	Light violet at the end of 5 hours.
3.	1:500	Nearly colorless at the end of 2 hours.
4.	1:1000	Colorless in about 15 minutes.
C.	......	Violet at the end of 5 hours.

It would seem from these results that, as already pointed out by Weigle and Merkel, the action of malt diastase is aided by the presence of formaldehyde. This, however, is due to the inhibition of bacterial growth by the formaldehyde. A glance at the tables shows that a solution of the ferment in water soon undergoes decomposition; after such a solution has stood for two or three days it is able to convert little if any of the starch. If formaldehyde is present in the proportion of

1:500 or 1:1000, the ferment does not decompose; on the other hand, it does not seem to be affected by the formaldehyde, for it is as active at the end of three weeks as when fresh.

CONCLUSIONS.

The following general conclusions may be drawn from the preceding work:

Fibrin is altered by formaldehyde and is then less easily digested by pepsin and by trypsin. Papaïn is apparently unable to digest fibrin even when this is exposed to very weak formaldehyde (1:1000) for a very short time.

The casein of milk, on contact with formaldehyde, undergoes rapid alteration and is as a result not coagulated by rennet, or but very slowly. Such altered casein, like similar fibrin, is not readily digested by the proteolytic ferments. The longer the formaldehyde acts on casein and on fibrin the more marked is the result.

Pepsin is not affected by a one per cent solution of formaldehyde, even when the mixture has stood for four weeks. Even a five per cent solution of formaldehyde acting for three weeks has no effect on pepsin. Contrary results obtained by others are due to an alteration of the fibrin by the formaldehyde. A putrid solution of pepsin in distilled water one month old digests fibrin as readily as a fresh solution.

Rennet is not affected even by a four per cent solution of formaldehyde acting for several weeks. The absence of coagulation at times is due to the action of formaldehyde on the casein of the milk and not on the rennet ferment.

Papaïn is very quickly altered by formaldehyde, even in very dilute solution. Moreover, it is unable to digest fibrin that has been exposed to the action of a very dilute solution of formaldehyde for a short time.

Trypsin is altered by formaldehyde to such an extent that digestion of fibrin will not take place, or but very slowly. The extent to which trypsin is affected by formaldehyde depends largely upon the amount of organic matter present, as well as on the amount of ferment in the solution.

Amylopsin is not destroyed by very dilute solutions of formaldehyde, but stronger solutions decrease the activity of the ferment, and if used in sufficient concentration will destroy it completely.

Ptyalin, like the diastatic ferment of the pancreas, is not destroyed by dilute solutions of formaldehyde. If the latter is used in rather strong concentration and allowed to act for some time it will destroy the ferment. The action of formaldehyde is more rapid and more marked at a slightly elevated temperature than at ordinary room temperature.

Malt diastase, unlike the diastatic ferments of the saliva and pancreatic solution, is not destroyed by formaldehyde when this is used in moderate amount and at ordinary temperature. Unlike pepsin, a solution of malt diastase readily undergoes decomposition on standing even for one or more days. This destruction is undoubtedly due to bacteria since it does not take place when formaldehyde is present. Consequently the favoring action which formaldehyde apparently exerts on diastase really consists in the inhibition of the growth of micro-organisms, and hence the diastase is protected against decomposition.

A SECOND CASE OF GONORRHŒAL SEPTICÆMIA AND ULCERATIVE ENDOCARDITIS WITH OBSERVATIONS UPON THE CARDIAC COMPLICATIONS OF GONORRHŒA.*

By WILLIAM SYDNEY THAYER, M. D., AND JESSE WILLIAM LAZEAR, M. D.

PLATE I.

Cardiac complications of gonorrhœa with or without coincident or preceding arthritis, while not of frequent occurrence, are by no means so rare as has, even within recent years, been supposed. The literature shows over a hundred cases in which a diagnosis of gonorrhœal endo-, peri- or myo-carditis has been made, and during the last several years considerable attention has been attracted to the subject by the accumulation of evidence demonstrating the fact that many of these complications are due to actual local infections with the gonococcus.

It was the good fortune of Blumer and one of the writers to observe a case of gonorrhœal ulcerative endocarditis in 1895 and to succeed for the first time in obtaining the gonococcus in pure culture during life from the circulating blood; moreover organisms showing all the characteristics of gonococci were demonstrated in the lesions upon the affected valves, thus furnishing definite proof of the possibility of the existence of a true gonorrhœal septicæmia and endocarditis.

This case, which has already been reported,† it may be well to summarize:

The patient, a woman 34 years of age, entered the Johns Hopkins Hospital, April 25, 1895. Her family and personal history were nega-

* This case was reported at the Twelfth International Medical Congress at Moscow in August, 1897.

† *Arch. de méd. expér. et d'anat. pathol.*, 1895, vii, 701; also, *The Johns Hopkins Hospital Bulletin*, 1896, vii, 57.

tive, excepting that for three months she had had rheumatism off and on in various of her joints. From the beginning of her rheumatism she had complained of weakness and dyspnœa on exertion. A few days before her entry into the hospital she had a severe chill and took to bed. On entrance there were well-marked signs of mitral stenosis. During the period of her sojourn in the hospital there was irregular fever associated with severe chills. The blood showed throughout a well-marked leucocytosis; the urine contained a trace of albumin and the sediment contained occasional casts. The patient grew rapidly feeble and died May 16.

The diagnosis of ulcerative endocarditis having been made during life, cultures were taken from the blood on several occasions. These cultures were made by Dr. Blumer according to the method of Sittman. The blood, taken from the median basilic vein by a sterilized syringe, was mixed with melted agar which was immediately plated. Large quantities of blood were used, so that the medium contained at least one-third blood. The first culture, taken May 4, was negative, but in the cultures of May 7 and 12 the plates showed very minute white colonies representing a pure culture of small biscuit-shaped diplococci which failed to grow on transmission to agar-agar, gelatine, potato, bouillon or litmus-milk. These organisms were decolorized entirely by Gram's method.

The autopsy confirmed the diagnosis made during life, revealing an extensive ulcerative and vegetative endocarditis of the mitral valve. In the thrombi upon the valve were found large numbers of diplococci having all the morphological and tinctorial characteristics of gonococci. At the time of autopsy there were unfortunately no media at hand suitable for the cultivation of gonococci, and implantations, made upon agar-agar and ox's blood serum, from the heart's blood, valves, liver, spleen, lungs and kidneys, were entirely without result; it should be stated that in these post-mortem cultures but a small quantity of the heart's blood was mixed with the agar. Inoculation of a mouse with a piece of thrombus from the valves was without result.

The characteristic appearance and disposition of the cocci, their decolorization according to the method of Gram, their failure to develop upon ordinary media, and finally their growth on two occasions during life upon a medium practically the same as that recommended by Wertheim, leaves, it seems to us, little doubt that this was a true gonococcal infection. Similar organisms were found after death in the vagina and uterus.

Certain reviewers have been inclined to doubt the complete reliability of this observation. Thus, Fraenkel* asserts that "because during life a gonorrhœal affection was not discovered in the patient despite careful search, and cultures of the observed microörganism were not made on human blood serum or Wertheim's serum agar, the observation cannot be considered as entirely free from criticism." We confess that we cannot see the justice of Fraenkel's observations. It is well known to gynaecologists that gonorrhœal affections often exist in women without being recognized by the ordinary methods of examination. As was stated in the previous communication, we had not thought during the life of the patient of the possibility of the case being one of gonorrhœal infection, and the vaginal secretion was not examined. But after death characteristic gonococci, answering to all tinctorial and morphological characteristics, were found both in the vagina and the uterus. Moreover, the medium upon which the successful cultures were twice obtained during life—the mixture of blood fresh from the veins with melted agar—was essentially similar to the human blood-serum agar of Wertheim. Upon this medium the organism grew; upon all ordinary media it failed to reappear.

Shortly after the publication of the foregoing case we observed a second instance of endocarditis and septicæmia of undoubtedly gonorrhœal nature.

J. K., aged 19, a day laborer, unmarried, a native of Germany, was admitted to the Johns Hopkins Hospital on February 5, 1896, complaining of fever and weakness.

Family history.—Father died with dropsy; mother, one brother and two sisters living and well; several brothers and sisters died in infancy.

Personal history.—There is no history of the ordinary diseases of childhood. He has never had any severe infectious diseases; is sure that he has never had rheumatism or scarlet fever, stating that he has always been a healthy man. He drinks beer in moderation.

Present illness.—The patient contracted gonorrhœa for the first time six months ago. Several weeks after the onset he began to suffer from chilly sensations, fever and general weakness. Toward the end of November he began to have violent chills, occurring usually in the morning hours; these were followed by fever and profuse sweating.

* *Hygienische Rundschau*, 1896, vi, 254.

Under treatment the shaking chills disappeared, but the fever continued, becoming, however, more irregular. He has grown progressively weak and pale, and for two weeks before entry there has been œdema of the feet and ankles.

Physical examination.—The patient is very dull and drowsy. He is a large well-nourished man; lips, mucous membranes and skin extremely pale; pulse large, but of low tension, 108; respiration 30; T°, 103.4°. Lungs, clear throughout. *Heart:* Point of maximum impulse in the 4th space just inside the mamillary line. Relative dulness begins in the 3d interspace and is not increased to the right. Absolute cardiac dulness is obliterated by pulmonary resonance. On auscultation the first sound at the apex is booming and prolonged; there is no actual murmur. Passing toward the base a soft systolic murmur becomes audible; most marked in the pulmonic area. The second pulmonic sound is a little sharper than the second aortic. *Liver:* hepatic flatness begins at the 7th rib in the mamillary line, the lower border being palpable, 6.75 cm. below the costal margin. *Spleen* is greatly enlarged, flatness above beginning at the 7th rib, while the lower border is palpable 9.5 cm. below the costal margin. *Abdomen:* full, bulging a little in the flanks, tympanitic in the elevated, flat in the dependent parts; well-marked movable flatness. The left knee-joint contains an excess of fluid, being distinctly swollen and fluctuating. No redness or tenderness. No tenderness or irregularities on any of the long bones. Moderate enlargement of the inguinal glands. Slight œdema of the feet and ankles. There is a thick, purulent urethral discharge showing characteristic gonococci. The *blood* contains no malarial parasites or pigment. There is a moderate poikilocytosis. Red blood corpuscles, 2,292,000; colorless corpuscles, 9,000.

Urine: reddish amber; acid; 1015; no sugar; albumin, 0.1 per cent. Sediment: considerable; whitish; microscopically, numerous pus cells, usually separate, not in clumps; red blood corpuscles; small round cells about the size of leucocytes with single nuclei; numerous hyaline and granular casts with adherent pus and degenerated epithelial cells; epithelial casts; pus casts.

The patient remained in the hospital but nine days, during which time the temperature ranged between 99.6° and 103.3°. The urine was somewhat reduced in quantity, averaging a little under 1000 cc. in the 24 hours. The specific gravity ranged between 1013 and 1015, while the amount of albumin and the character of the sediment continued about as noted above.

11/ii/96. *Examination of the blood:* Red blood corpuscles, 2,283,000; colorless corpuscles, 14,250; hæmoglobin, 45 per cent. Dried specimens prepared according to Ehrlich's method showed slight variations in the size of the corpuscles, moderate poikilocytosis; a few nucleated red corpuscles, no malarial parasites. Differential count of leucocytes: small mononuclear leucocytes, 4.8 per cent; large mononuclear and transitional leucocytes, 2.6 per cent; polymorphonuclear neutrophilic leucocytes, 92.6 per cent; eosinophilic leucocytes, none.

The direct sequence of the symptoms upon the gonorrhœa suggested to us the possibility that we might be dealing with a gonorrhœal pyelo-nephritis and possibly with a general septicæmia, and cultures were taken on two occasions by Dr. Lazear from the circulating blood by the same method adopted in the previous case. These cultures were without result in both instances.

The patient was kept in bed, placed upon a milk diet, diuretics and iron. On February 14 he left the hospital, objecting to the strict régime.

March 9 the patient re-entered the hospital, having grown steadily worse.

10/iii/96. *Physical examination.*—The patient was extremely sallow, pale; tongue dry and fissured; pulse 108; moderate œdema of the dependent parts; slight puffiness of the face. The point of maximum cardiac impulse had moved outward and downward to a point in the 5th space slightly outside of the mamillary line, while a soft systolic murmur, which was not present on the former admission, was now to be heard all over the cardiac area, loudest at the apex. The second sounds at the base of the heart were not loud, but were of normal relative intensity; no accentuation of the second pulmonic sound.

The urethral discharge had almost disappeared.

The anæmia had increased, the *blood count* showing on 10/iii/'96: red blood corpuscles, 1,920,000; colorless corpuscles, 8500; hæmoglobin, 18 per cent.

The *urine* of the same date was of a pale but distinctly smoky color; acid; sugar absent; albumin 0.4 per cent. Sediment, abundant; microscopically, many pus cells scattered throughout the field, not arranged in clumps; many small round cells about the size of leucocytes with single nuclei; many red blood corpuscles, some "shadows," others crenated, others fairly well preserved. Numerous fatty degenerated epithelial cells, somewhat larger than pus cells; some small agglomerations of free yellow fat drops; occasional compound granular cells; casts

extremely abundant; hyaline, finely and coarsely granular with adherent pus and red blood cells; occasional fatty casts and blood casts; many epithelial and pus casts; occasional extremely large, slightly yellowish, typically refractive, waxy casts with broken ends; diazo-reaction absent.

The patient grew rapidly worse; the anæmia increased, the *blood* on the day of death showing: red blood corpuscles, 1,896,000; colorless corpuscles, 18,000; hæmoglobin, 18 per cent.

The *urine*, averaging little over 600 cc. for the 24 hours, was almost suppressed during several days before death. The albumin increased up to nearly 0.5 per cent, while the number of fatty, blood, waxy, epithelial and pus casts increased. On March 23 the paient became extremely dull and drowsy.

23/iii/96. (Professor Osler.) "For the past few days the temperature has been lower, not above 100° since the 19th; no change in the general condition. Pulse about 100; drops a beat occasionally; of low tension.

Heart: apex beat is diffuse during expiration; well seen in the 5th space and a little outside the nipple line. Cardiac impulse is visible in the 4th space inside the nipple. A diastolic shock can be felt at the apex; both sounds are audible; no murmur. Over the entire praecordium there is a to-and-fro superficial pericardial friction murmur, the maximum of which is at the 5th left cartilage; it is well heard at the ensiform cartilage; not heard above the level of the 3d rib; no especial accentuation of the pulmonic second sound."

On the 24th the patient developed a well-marked petechial eruption.

25/iii/96. (Dr. Thayer.) "The patient is lying on his right side; very drowsy and dull. Respirations 15 to the minute, rather deep and noisy; pulse 21 to the quarter; of low tension. Face puffy; pupils not contracted; general anasarca.

Heart: diffuse heaving over the 4th and 5th interspaces just inside the nipple; the point of maximum cardiac impulse is not to be sharply differentiated; flatness does not pass the left sternal margin; begins at about the 4th space. At the apex the first sound is reduplicated and followed by a soft systolic murmur, while in connection with this there is a soft superficial to-and-fro rub. Over the body of the heart the sounds are considerably masked by this friction rub. *The second pulmonic sound is, however, not accentuated.* On the trunk and arms and occasionally on the legs are numerous small petechial spots, the largest scarcely larger than the head of a pin."

On March 21 the patient began to suffer from diarrhœa, the movements

becoming gradually more frequent and fluid. During the afternoon of the 25th the respiration became more stertorous; the patient lapsed into a condition of complete coma and died at 6.30 P. M.

Bacteriological examination during life.—March 22, 1896, cultures were made by Dr. Lazear in the following manner: 2 cc. of blood were drawn from the median basilic vein by a hypodermic syringe previously sterilized by boiling. The skin was, as far as possible, sterilized and every antiseptic precaution was used. The blood was mixed with 1 cc. of melted nutrient agar and the mixture poured into a Petri dish and allowed to harden. It was kept in a thermostat at 35° C. At the end of 24 hours no growth was visible. At the end of 48 hours there began to appear colonies half the size of a pin head, granular in appearance with somewhat irregular borders. These colonies were made up of cocci arranged usually in pairs. The cocci were of biscuit or kidney shape, their flattened sides being adjacent. They stained well with the ordinary basic dyes and decolorized by Gram's method. Transplanted upon human blood-serum agar by a smear upon the surface there developed a fair number of colonies similar to the above, consisting of diplococci having the same morphology and tinctorial characteristics. Transplanted to ordinary agar there was a growth of a few fine colonies of the same diplococci. On gelatine, ox's blood serum and bouillon there was no growth. At the end of ten days the organisms had all died out upon the original plates.

On March 24 and 25, plate cultures were made by the same method, and on each occasion there was an abundant growth, in pure culture, of diplococci identical with those obtained upon the first plates, behaving in the same manner with regard to stains and in their growth upon the various culture media.

On the basis of these positive cultural experiments and the clinical history of the case, the patient was brought before the class on March 25 as an instance of general gonococcal septicæmia with endo- and pericarditis.

Autopsy, March 25, by Professor Flexner.

Anatomical diagnosis.—Gonococcal septicæmia; subacute gonorrhœa; subacute ulcerative and vegetative, tricuspid endocarditis caused by the gonococcus; subacute splenic tumor; chronic passive congestion of the liver; subacute hæmorrhagic and glomerular nephritis; acute sero-purulent pleurisy and pericarditis due to the gonococcus; pulmonary infarct.

The following is a summary of the pathological record:

On opening the abdomen nothing remarkable was to be made out

except the much enlarged spleen, adherent in its upper part to the diaphragm.

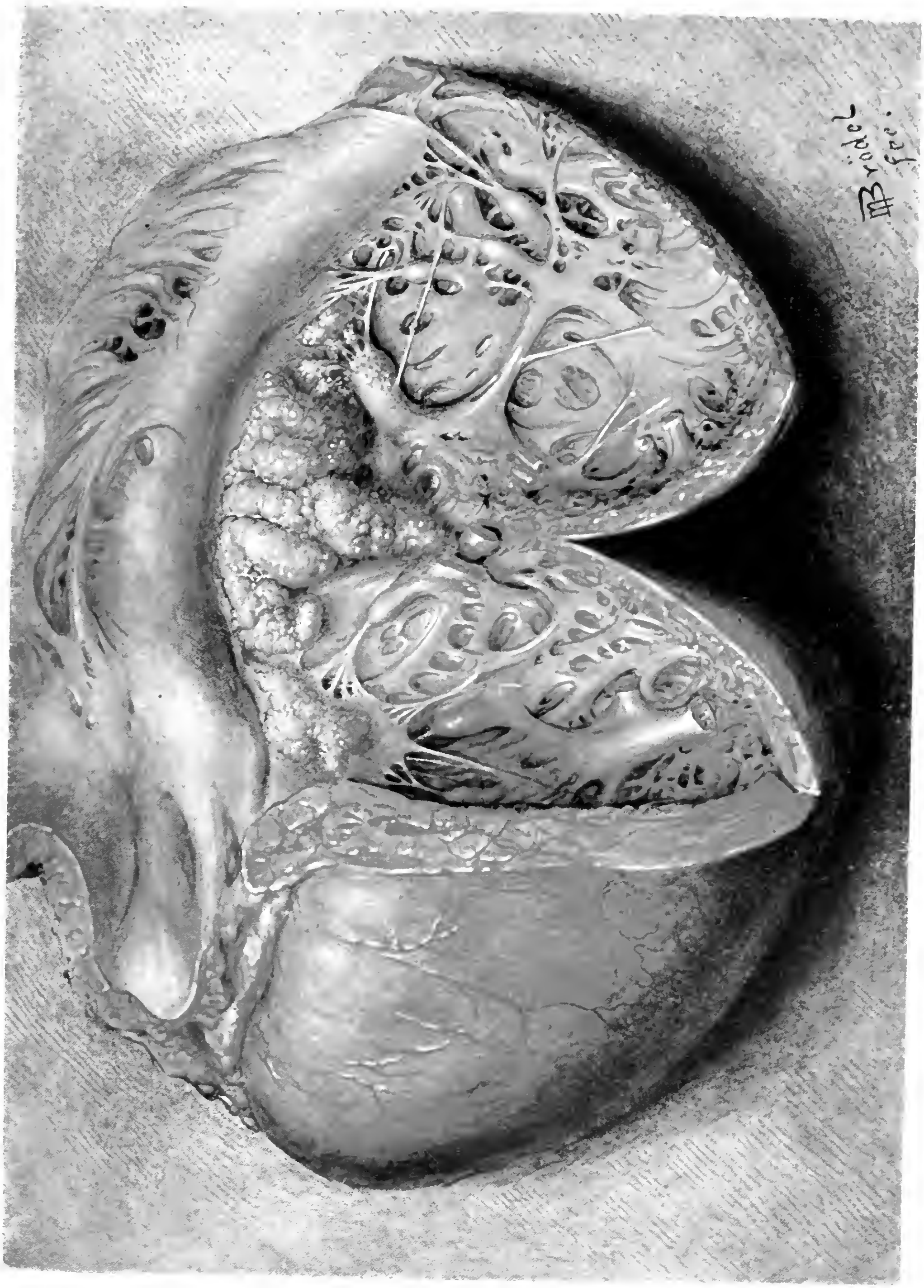
Pleurae and lungs.—The right pleural cavity is free from adhesions and contains about 800 cc. of slightly turbid, yellowish fluid with large flakes of fibrin. On the pulmonary pleura there are small flakes of fibrin and beneath this are many punctiform ecchymoses. The left lung is bound down along its posterior border by firm adhesions. In the left pleural cavity are 550 cc. of fluid containing somewhat less fibrin than on the other side. There are less fibrin and fewer ecchymoses on the visceral pleura than on the right side.

In the middle of the left lower lobe at its inferior border there is a circumscribed, triangular area of infarction 1½x1½ cm. in extent; opaque, firm, of a brownish-yellow color. The surrounding tissue is congested. On section both lungs are of a light salmon-red color and œdematous. The bronchi are congested. The pulmonary arteries are free and practically normal in appearance. The anterior edges of the lungs are emphysematous.

Pericardium and heart.—The pericardial cavity contains about 300 cc. of turbid yellow fluid with fibrinous flakes. In the dependent portions the fluid is thick and puriform. The surface of the peri- and epicardium is congested, ecchymosed, and covered by a granular deposit. The weight of the heart and pericardium is 680 grammes.

The right ventricle and auricle are dilated and contain fluid blood and partly decolorized clots. The endocardium of the right auricle is delicate, although near the obliterated foramen ovale beneath the endocardium are two or three slightly elevated minute opaque points.

The tricuspid valve is the seat of an extensive thrombus, occupying the entire middle segment, with the exception of its base, and to a less extent the two remaining segments (see Plate I). The thrombus attached to the middle segment is firmly united to the valve at a distance of 5 mm. from its attachment to the auriculo-ventricular ring and projects into the cavity of the ventricle. At the site of attachment of the thrombus the substance of the valve is destroyed. The thrombus presents an irregularly convoluted appearance, and for description may be divided into three distinct portions: The central portion, which is largest, measuring 4.5x2 cm. x4.5 mm. in thickness, has in general a conical shape with its base at the valve and its apex projecting into the ventricle. To the left of this is a second mass 18x22 mm. in extent, almost quadrangular in form but irregular in contour. The remaining mass to the right is about one-half the size of the last. This thrombus



mass is attached at certain points to the endocardium of the right ventricle. Fully one-half of the chordæ tendineæ of the segment of the valve are ruptured and their free ends are covered with grape-like clusters of thrombi. Small miliary vegetations are on the papillary muscle to which these are attached and on a moderator band which extends from the papillary muscle to the mid-portion of the left segment of the valve. The anterior surface of the right segment of the valve is the seat of two thrombus masses projecting into the auricle; they average about 12x10 and 12x6 mm. The pulmonary artery and valves are normal in appearance.

The aortic and mitral valves and the endocardium of the left ventricle are entirely normal. In the lymphatics along the course of the vessels in the epicardium on the right side there are minute discoid nodules similar to those described in the endocardium of the auricle.

The tricuspid orifice measures 16 cm.; length of the right ventricle 12.5 cm.; thickness 5-7 mm. The mitral valve measures 12 cm.; length of the left ventricle 8 cm.; thickness 15 mm. The cardiac muscle is pale and fairly firm.

The *spleen* weighs 840 grammes; dimensions, 21x13x6 cm.; capsule wrinkled; on section the Malpighian bodies very prominent, the trabeculæ visible; the pulp of an opaque grayish-red color, the consistence moderately firm.

The *liver* weighs 2450 grammes, and is the seat of fairly well-marked chronic passive congestion.

Kidneys.—The kidneys weigh together 670 grammes. Dimensions: right, 14x9.5x4; left 15x8x4. They are swollen, the capsule adherent in places; the surface mottled, of an opaque, grayish color with many punctiform hæmorrhages. The cut surface appears swollen and œdematous. The striæ are obscure; the glomeruli visible, but pale. Numerous elongated hemorrhages—9 to 10 mm. long—are visible in the cortex. The pyramids are hyperæmic; the vessels of the mucous membrane of the pelvis are also somewhat congested and a few small hæmorrhages are visible.

The *ureters* are not enlarged; their mucous membrane shows the same condition as that of the renal pelvis.

Bladder.—The vesical mucous membrane is pale, with the exception of the trigonum, where it is moderately congested. About the orifices of the ureters there are a few small hæmorrhages. The neck of the bladder and the prostatic portion of the urethra are somewhat hyperæmic.

Gastro-intestinal tract.—The stomach and intestines show nothing noteworthy.

Pancreas.—A number of small opaque areas of fat necrosis are to be made out in the fatty capsule of the pancreas. Teased preparations show in the necrotic areas groups of fat cells containing finely granular bodies. Treated under the microscope with concentrated sulphuric acid, gas bubbles may be seen to arise and soon fine crystals of calcium sulphate appear.

Bacteriological examination.—Cover-slips from the pleural and pericardial exudates and from the vegetations upon the heart valves showed large numbers of cocci in pairs. They were excessively numerous upon the surface of the tricuspid valve. These cocci were in almost all instances included within polymorphonuclear leucocytes which were numerous. The form of the cocci was, as a rule, typically biscuit-shaped, and at times, though rarely, two pairs lay side by side, suggesting a tetrad arrangement. The cocci stain readily in the usual aniline dyes, but are quickly and uniformly decolorized by Gram's method.

A cover-glass specimen from the spleen showed one pair of cocci; others were not found. Cover-slips from the kidney, renal pelvis and urinary bladder were negative.

In the urethra, among a variety of bacilli, definite intracellular biscuit-shaped diplococci which decolorize by Gram were found.

Cultures.—Cultures were made at the autopsy from the various local exudates, the heart's blood and the organs as follows:

(*a*) Upon Loeffler's blood serum, prepared: (1) from human blood; (2) from bullock's blood; (3) from dog's blood.

(*b*) Upon agar-agar. Only a few tubes of the human serum were on hand and these were used for the local exudates and blood. Upon those from the pericardium, tricuspid valve and heart's blood delicate growths were obtained which consisted in part of confluent minute colonies, in part of small, almost point-like, grayish-white, slightly elevated colonies. Microscopically these colonies were composed of diplococci, readily decolorizing by Gram, and resembling in every way, except perhaps in the feebleness of their growth, the organisms isolated during life. No growths whatever were obtained upon bullock's serum, dog's serum, or plain agar-agar.

Transplantations from the 48-hour old growths from the human blood serum on to ordinary agar, swine-liver agar (Livingood), fœtus agar (Flexner) were negative. As no human serum remained, transplantations entirely failed.

Microscopical examination.—A histological study of the organs was made by Professor Flexner, to whom we are indebted for the following report: The description of the histological changes is confined to those of the kidneys, the liver, the spleen and the cardiac valves, these being the parts chiefly affected. The tissues were hardened in alcohol and Zenker's fluid, and the specimens stained by hæmatoxylin and eosin, Weigert's fibrin stain, and methylene-blue.

Kidney.—Throughout the cortex there is a general increase of connective tissue, uniformly distributed and particularly well marked between the tubules. This tissue is fibrillated, œdematous and not particularly rich in cells. The chief lesions affect the glomeruli and the labyrinthine tubules. The least affected glomeruli completely fill the capsule of Bowman; the number of cells within the glomerular capillaries is increased, this increase being due to an excess of polymorphonuclear leucocytes. The capillary walls are distinct, thicker than normal, hyaline or slightly fibrillated in appearance. The more abnormal glomeruli show, in the first place, thrombosis of groups of capillaries by material presenting characters unmistakably indicative of fibrin. This material within the capillaries appears as a delicate network and may be limited to a single loop of a capillary or occupy a group of loops; it does not completely occlude the vessels. Outside of the capillary walls there is an increase in the number of cells within the glomerular space. These cells are partly of an epithelioid type, being doubtless derived from the capsular and glomerular epithelium, and partly leucocytes. Deposited within and intimately mixed with these cells a fibrinous material, which appears often as a crescentic band, dips down between the lobules of the glomeruli. It is in part distinctly fibrillated, in part dense and hyaline, and everywhere gives a sharp staining reaction for fibrin. Its association with the cells within the capsular space is of the most intimate character. In specimens stained with hæmatoxylin and eosin it takes a vivid red stain. In not a few situations there is evidence of a proliferation of cells clearly derived from the capsular epithelium, and, although less certainly marked, there is in our mind little doubt that a similar increase of cells is taking place within the capillaries themselves.

As has been stated, leucocytes occur abundantly in the capsular spaces; these are derived from the glomerular capillaries and may be seen in the act of migration through the capillaries into the space. Of particular interest is the passage of leucocytes from the capsular space through the capsule of Bowman into the interstitial tissue in which these

cells are increased. The uriniferous tubules contain also large numbers of polymorphonuclear leucocytes; in some places completely occluding the lumen of the tubes, forming definite leucocytic casts. The epithelium, especially of the secreting tubules, is much degenerated, even necrotic, and in some places evidently proliferating, as is evidenced by multinucleated cells in certain of the tubules. The degeneration of the epithelium is partly fatty and granular, but largely of the hyaline variety, to which change can be attributed much cast material and definite casts occupying the tubules. Red blood corpuscles are rarely found within the tubules. The essential lesion is a sub-acute glomerular and intracapillary nephritis. Bacteria were not discovered in this organ.

Spleen.—The connective tissue framework of the spleen is thickened. The new tissue is of a semi-fibrillated character. The blood-vessels of the pulp are diminished in size and apparently also in number. The splenic elements proper are also reduced in number, but there are scattered irregularly throughout the spleen in greatly increased number polymorphonuclear leucocytes, and within the venous sinuses groups of hyaline bodies and single hyaline bodies, globular in shape, varying in size from a red blood corpuscle to one of the largest white cells there present.

The follicles are enlarged and very distinct, many of their cells being swollen and in process of division; they are infiltrated with polymorphonuclear leucocytes, the nuclei of many of which show the greatest irregularity in form. Nuclear fragments are scattered sparsely and irregularly throughout the spleen; for the most part they are not enclosed within other cells.

Liver.—The connective tissue is not increased; the central veins are much dilated and the central portions of the lobules hyperæmic. This congestion varies in different lobules, being in some very marked, with corresponding atrophy of the liver cells. There is an interesting hyaline metamorphosis of some of the liver cells. Those peripherally placed in the congested areas show this change best, and in these the nuclei have become small, contracted and deeply staining. In parts of the liver distant from the congested areas the hepatic cells are swollen, fatty, and free from the hyaline change described, excepting where an occasional cell, more or less loosened from the rows of liver cells and perhaps lying in the capillary, is thus affected. There is a general increase in the number of leucocytes within the blood-vessels.

Heart.—Sections through the tricuspid valve include chiefly the thrombus, which consists of masses of blood platelets, of fibrin and of

included leucocytes. No bacteria appear in sections stained by Gram's or Weigert's method.

The pericardium shows, besides a superficial fibrinous exudate containing leucocytes, a considerable inflammatory infiltration of the subjacent fibrous tissue and a proliferation of the fixed tissue cells. The epithelial covering is in active proliferation. In some of the leucocytes in the pericardial exudate Dr. Lazear was able to demonstrate gonococci.

A further study of the heart valves was made by Dr. Lazear: The tissues were hardened in alcohol and in Zenker's fluid, and embedded in celloidin and paraffin. For the bacteriological study they were stained with Loefller's and Unna's methylene-blue, Ziehl-Neelsen carbolic fuchsin, Stirling's gentian violet and by Gram's method. For histological study they were stained with hæmatoxylin and eosin and Weigert's fibrin stain.

Tricuspid valve.—The connective-tissue structure of the valve shows areas of necrosis. In places the nuclei are merely swollen. There are also irregular areas extending throughout the valve in which the nuclei have entirely disappeared, leaving a homogeneous material which stains with eosin. Extending into the substance of the valve are numerous spaces filled with leucocytes, among which are a few large phagocytic cells, with large irregularly shaped nuclei and containing red blood corpuscles.

The vegetations are made up of granular masses of platelets surrounded by fibrin and a thick layer of leucocytes. Leucocytes are present also within the masses of platelets, either scattered or occupying spaces.

Upon the surface of the valve and the vegetations is a layer composed of leucocytes and red blood corpuscles with strands of fibrin forming an irregular network through it. The same material occupies the interstices between the vegetations. Many of the leucocytes in this layer and in the more central portions contain micrococci with the typical biscuit shape and other morphological characters of gonococci and completely decolorizing by Gram. Extracellular gonococci are present in the central portion of the thrombi but not in the superficial layers.

At the line of junction of the central platelet mass and the leucocytic layer is a long line of globular bodies varying in size from that of a leucocyte to ten times this size. These bodies are made up of dense masses of gonococci.

There can, it appears to us, be no doubt as to the nature of the organisms obtained in pure culture from the blood during life and

from the affected valves, heart's blood and pericardium after death. This case and that previously reported by Dr. Blumer and one of the authors (Thayer) are the first two recorded instances in which absolute proof of the gonococcal nature of the general infection has been obtained.

The case presents several interesting and unique features. While the diagnosis of ulcerative endocarditis and gonorrhœal septicæmia was made during life, the exact anatomical lesion—the remarkably extensive affection of the tricuspid valve—was not at this time suspected. Much of the thrombus upon the valve may have been of relatively recent formation, but older changes, probably of several months' duration, were clearly present. It is the first instance in which a pure tricuspid lesion has been found in a gonorrhœal endocarditis. It is not uninteresting that while the diagnosis was not made clinically, it was particularly noted that, in association with the systolic murmur, there was no accentuation of the second pulmonic sound, a fact which might well have excited our suspicion.

The changes in the kidney are of especial interest. Clinically the case presented the features of a grave acute nephritis with anæmia, anasarca, ascites and finally uræmic coma. The urine showed, besides the large quantity of albumin, the blood casts and the epithelial cells, so large an amount of pus that a diagnosis of probable pyelitis or pyelo-nephritis was made. Especially interesting is the fact that during life casts consisting entirely of polymorphonuclear leucocytes were repeatedly observed. The absence of gross collections of pus in the kidney or in the pelvis was a surprise at the time of the autopsy. The microscopical examination of the kidney, however, revealed the source of this pus in the unquestioned evidence of the passage of numerous leucocytes directly through the capillaries of the glomeruli into the glomerular spaces and the urinary tubules. The extensive thrombosis of the glomerular capillaries and the accumulations of fibrin in the glomerular spaces form a very remarkable picture. How this special localization of these changes in the kidney and the relative freedom of the liver from those degenerative processes so common in general septicæmia, viz. focal necroses, may be explained is rather

an interesting question. May it perhaps be true that the renal changes were due to the direct deleterious action of the gonococci or their products in the process of elimination through the urine?

That the gonococcus must be recognized as a true pyogenic organism capable of giving rise to the gravest local and general septic complications has been abundantly proven by recent observations. That cystitis, epididymitis, spermatocystitis, prostatitis and peri-urethral abscesses in man, metritis, vulvo-vaginitis, salpingitis, and peritonitis in woman, may owe their origin solely to the presence of the gonococcus has been clearly demonstrated. And further, suppurative processes in remote parts are now known to be occasionally due to a pure gonorrhœal infection. Horwitz and Lang* found the gonococcus in an ulcer upon the back of the hand, while its presence in joint fluids in cases of gonorrhœal arthritis has been demonstrated by many observers. Within the past 15 months Dr. Young has obtained the gonococcus in pure culture in 7 instances of gonorrhœal arthritis in the Johns Hopkins Hospital, demonstrating that the arthritis in a large proportion of instances represents a true local bacterial infection.

The gonococcus has also been obtained in pure culture from a number of instances of tenosynovitis (Jacobi and Goldmann,† Bloodgood and Young‡), from subcutaneous abscesses (Lang and Paltauf§ and Horwitz‖), from intramuscular abscesses (Bujwid¶), from pleural effusions (Bordoni-Uffreduzzi**), from the circulating blood (Thayer and Blumer,†† Thayer and Lazear‡‡), and recently by Young§§ from a case of general peritonitis following an acute gonorrhœal

* *Wiener klin. Wochenschr.*, 1893, vi, 59.
† *Beiträge z. klin. Chir.*, 1894, xii, 827.
‡ Unpublished observations in the Johns Hopkins Hospital.
§ *Arch. f. Derm. u. Syph.*, 1893, xxv, 330.
‖ *Wien. klin. Woch.*, 1893, vi, 59.
¶ *Centrlb. f. Bakt.*, 1895, xviii, 435.
** *Deutsche med. Woch.*, 1894, xx, 484.
†† Op. cit.
‡‡ *Med. Record*, N. Y., 1897, lii, 497, and present article.
§§ Unpublished observation.

salpingitis. That in some instances, therefore, the gonococcus itself should be the cause of endocarditis, pericarditis, and myocarditis is by no means remarkable. Our own experience during the last several years, together with a study of the literature, indicates that the cardiac complications of gonorrhœa are more frequent than has generally been supposed.

Endocarditis is by far the commonest of the cardiac complications of gonorrhœa. Gurvich* has collected 110 instances in 77 of which the cases are sufficiently well reported to allow of definite conclusions. From the more recent literature it is possible to add some ten or a dozen more cases to those of Gurvich.

In the majority of these cases the cardiac complications have been preceded by an arthritis. In a considerable number of instances, however, joint symptoms have been entirely absent, while in several cases evidences of endo- or pericarditis have appeared before the development of joint manifestations.

Pericarditis is a much less frequent complication. We have been able to collect but 17 positive cases in the literature.

Myocardial changes have been demonstrated in the majority of the cases of acute ulcerative endocarditis of gonorrhœal origin which have come to autopsy, the most satisfactorily studied instance being that of Councilman, where there was no affection of the endocardium.

For a satisfactory study, however, of the cardiac complications of gonorrhœa one must turn to the cases which have been observed anatomically as well as clinically. We have collected 32 instances of gonorrhœa with fatal cardiac complications where there were satisfactory pathological notes. Several of those included in other classifications have been omitted because of insufficient data. Of these 32 cases 31 were instances of ulcerative endocarditis with or without marked pericardial or myocardial affection; one, that of Councilman, was an instance of peri- and myocarditis alone.

Gonorrhoeal endocarditis.—These cases considered from a pathological standpoint may be divided into five classes:

* *Russk. arch. patol., klin. med. i. bakt.*, 1897, iii, 329.

(1) The first class includes six cases, those of Bourdon,* Desnos,† Schedler,‡ Draper,§ Fleury,‖ and His.¶

In these instances, although the history clearly shows the association of the process with gonorrhœa, no note is made with regard to bacteriological examination.

(2) The second class comprises ten cases, those of Martin,** Weckerle,†† Weichselbaum,‡‡ Ely,§§ Wilms,‖‖ Golz,¶¶ Keller,*** Zawadzki and Bregman,††† Babes and Sion,‡‡‡ and lastly an unpublished observation of our own, Case 32 of our series (p. 115). Here there existed mixed or secondary infections. In some instances organisms other than gonococci were obtained in pure culture; in others, strepto- and staphylococci were demonstrated microscopically. In several cases organisms morphologically similar to gonococci were found, while the actual sequence of the infection upon acute gonorrhœa is not to be doubted.

(3) The third class includes four cases, those of Rothmund,§§§ His,‖‖‖ Winterberg¶¶¶ and Fressel.**** Here the infection was probably purely gonococcal. In all of these cases organisms showing the morphological and tinctorial characteristics of gonococci were

* *Gaz. d. Hôp.* Par., 1868, xli, 1.

† *L'Union méd.*, 3s., 1878, xxv, 43; also, *Gaz. d. Hôp.*, 1877, l, 1067.

‡ "Zur Casuistik der Herzaffectionen bei Tripper." Inaug.-Diss., Berlin, 1880.

§ *Medical Bulletin*, Phila., 1882, iv, 81.

‖ *Journ. de méd. de Bordeaux*, 1883-84, xiii, 65.

¶ *Berl. klin. Woch.*, 1892, xxix, 993.

** *Rev. méd. de la Suisse Romande*. 1882, ii, 308, 352.

†† *Münch. med. Woch.*, 1886, xxxiii, 563, 582, 608, 622, 636.

‡‡ *Centralbl. f. Bakt.*, 1887, ii, 209.

§§ *Med. Record*, xxxv, 1889, 287.

‖‖ *Münch. Med. Woch.*, 1893, xl, 745.

¶¶ Ulceröse Endocarditis der Klappen der Pulmonalarterie bei gonorrhoischer Arthritis, Inaug.-Diss., Berlin, 1893.

*** *Deutsch. Arch. f. klin. Med.*, 1896, lvii, 387.

††† *Wien. med. Woch.*, 1896, xlvi, 313, 351.

‡‡‡ *Arch. d. Sc. Méd. de Bucarest*, 1896, i, 505.

§§§ Endocarditis ulcerosa, Inaug.-Diss., Zürich, 1889.

‖‖‖ Op. cit.

¶¶¶ Festschr. z. 25 Jähr. Jub. d. Vereins Deutscher Aerzte zu San Francisco, 1894, 40.

**** Inaug.-Diss., Leipzig, 1894.

demonstrated microscopically. Cultures, however, were not made. Other organisms were not found.

(4) The fourth group comprises six cases, those of Leyden,* Finger, Ghon and Schlagenhaufer,† Hale White,‡ Michaelis,§ Stengel || and Siegheim.¶ Here the proof of the purely gonococcal nature of the process is more nearly complete in that the observers made cultures upon ordinary media from the affected parts and from the circulating blood without obtaining positive results; while at the same time the microscopical demonstration from the affected regions of organisms showing all the morphological and tinctorial characteristics of gonococci would seem to form a fairly conclusive argument in favor of the existence of a pure infection by the gonococcus.

(5) Lastly, there remain five cases, those of Thayer and Blumer,** Dauber and Borst,†† Thayer and Lazear,‡‡ Rendu and Hallé§§ and Lenharz,|||| in which the evidence of the purely gonococcal nature of the complication may be considered as definitely proven.

In the first of these cases the gonococcus was obtained in pure culture twice during life and was found microscopically post-mortem in the affected regions.

In the case of Dauber and Borst a pure culture was obtained after death from the heart's blood of organisms concerning the nature of which the reporters were in doubt. Most subsequent observers have, however, recognized them as gonococci.

In the third instance, the writers were able to prove the purely gonorrhœal nature of the affection by obtaining gonococci in pure culture three times during life from the circulating blood, while Dr. Flexner obtained similar results post mortem from the affected valves, heart's blood, and pericardium.

* *Deutsch. med. Woch.*, 1893, xix, 909.

† *Arch. f. Dermat. u. Syph.*, 1895, xxxiii, 141, 323.

‡ *Lancet*, 1896, i, 533.

§ *Zeitschr. f. klin. Med.*, 1896, xxix, 556.

|| *Univ. Med. Mag.*, Phila., 1897, ix, 426.

¶ *Zeitschr. f. klin. Med.*, 1898, xxxiv, 526.

** Op. cit.

†† *Deutsch. Arch. f. klin. Med.*, 1896, lvi, 231.

‡‡ *Med. Record*, N. Y., 1897, lii, 497 (case reported in this communication).

§§ *Bull. et mém. Soc. méd. d. hôp. de Par.*, 1897, 3. s., xiv, 1325.

|||| *Berl. klin. Woch.*, 1897, xxxiv, 1138.

In the case of Rendu and Hallé gonococci were obtained in pure culture from the endometrium during life and demonstrated microscopically in the phlegmon about the elbow. After death they were obtained in pure culture from the thrombus upon the affected valves.

Finally the last link in the chain of evidence has been supplied by Lenharz, who, from a case of characteristic gonorrhœal endocarditis, in which pure cultures were obtained from the thrombi on the aortic valves, introduced a piece of softened thrombus into the human urethra. A characteristic gonorrhœa, with gonococci in the discharge, appeared on the fourth day.

Anatomical lesions.—As to the nature of the anatomical lesion there is little to say. Considering the 15 cases in which the pure gonococcal nature of the infection is probable, one finds that in all instances there were present the usual appearances of ulcerative endocarditis with extensive polypoid ehrombi and more or less actual destruction of the valves, often with aneurism formation and perforation. The localization of the affection, however, presents several points of considerable interest. In erdinary chronic endocarditis it is well known how infrequently the right heart alone is affected; thus, out of 300 autopsies on cases of endocarditis, Sperling found the lesions limited to the right heart in but 3 instances, or 1 per cent. Weckerle out of 846 autopsies on cases showing valvular cardiac lesions in Bollinger's laboratory found that the right heart alone was affected in 3.9 per cent of all cases.

It has, however, been shown that in cases of ulcerative endocarditis the liability of the right side to infection is considerably greater. Thus, while in 802 benign cases from the Munich statistics the right heart alone was affected in 3.24 per cent, in 44 cases of ulcerative endocarditis the percentage of affections limited to the right side was nearly 16 per cent (15.91 per cent). In our 31 cases the lesions were as follows:

Left heart:	aortic......	12
	mitral.....	6
	both.......	3
		21—67.7%

Right heart:	pulmonary......	7
	tricuspid.......	1
		8—25.8%

Both sides:	mitral, aortic, tricuspid......	1
	all four valves..............	1
		2—6.4%

This surprisingly high percentage of right-sided cardiac affections in our cases is interesting and difficult to explain. Considering the 15 cases in which the pure gonococcal nature of the infection is probable, we have the following table:

Left heart:	aortic.......	7	Right side:	tricuspid......	1
	mitral......	2		pulmonary....	2
	both	2			3—20%
		11—73.3%			

Both sides: all four valves......1-6.6%.

The remarkable high percentage of pure right-sided cardiac affections in these cases, even as compared with the Munich tables, is an interesting point. To attempt to draw definite conclusions from so small a number of instances might well be fallacious, but the fact is none the less worthy of reflection. Indeed, why the right side of the heart should be so much more liable to disease in ulcerative endocarditis is by no means perfectly clear. Another very interesting point is the fact that the aortic valves appear from our tables to be by far the most frequently affected. While this has been the case in those fatal instances which have come to autopsy, yet Gurvich's tables based upon 64 cases in which a diagnosis was made during life show appreciably different figures. His tables show:

Mitral valve	31	Pulmonary	2
Aortic	16	Mitral and tricuspid	2
Mitral and aortic........	13		

Age.—The age of the patients in the fatal cases varied between 19 and 51, while taking the larger statistics of Gurvich we find records of one instance at the age of 64 and another at 10. The majority of cases occurred, as might be expected, in early adult life.

Sex.—Of the 31 instances of fatal ulcerative endocarditis due to the gonococcus 23 were in men and 8 in women.

Time of onset.—There appears to be nothing particularly characteristic with regard to the time of onset of these cases with relation to the attack of urethritis, some, as in the case of Prévost, coming on almost immediately after the onset, others some weeks or months later. Some of the cases occurred with the initial attack of gonorrhœa, others in patients who had suffered once or twice before.

Complicating arthritis.—Arthritis preceded the cardiac affection in the majority of instances, though in a considerable number the cardiac complication occurred without or before the development of joint symptoms.

Symptoms.—The symptoms of gonorrhœal endocarditis appear to differ in no essential way from those of endocarditis of other origin. While many instances are reported in which the cardiac manifestations were slight and transient, the proportion of cases of a malignant nature which have so far been recognized is unusually large. It is not improbable, however, that this is due to the fact that the majority of milder cases escape notice. And it is well in this connection to remember how frequently the same is true in rheumatic cardiac affections.

In the fatal cases the symptoms are essentially similar to those in instances depending upon infection with other pyogenic organisms—an irregular intermittent or remittent fever, usually with severe rigors, profuse sweating and rapidly developing anæmia, albuminuria; in fact, the ordinary symptoms of a severe septicæmia. The duration of the attack in the 15 instances in which the pure gonococcal nature of the infection is probable, varied, as far as can be made out, from ten days to two months. The only exception to this rule was in our case, in which the symptoms of septicæmia lasted through a period of six months. From the consideration of these cases and of those others which were followed by recovery, it is rather difficult to see upon what Gurvich bases his conclusion that gonorrhœal cardiac affections pursue a milder course than those depending upon the other pyogenic cocci.

In a number of these instances the renal changes have been particularly severe. In several the patient died with the general appearances of a grave nephritis as in our second case. These changes are possibly to be explained by the special exposure of the kidney through the elimination of the gonococcus or its products which, in these instances of general septicæmia, occurs in all probability through the urine.

Pericarditis.—Pericarditis is a far less frequent occurrence than

endocarditis in gonorrhœal infection. In 7 of the 32 fatal instances there is a note of a pericardial complication. The cases of Weckerle and Golz were mixed infections. In Councilman's case the pericardial cavity was enormously distended, containing 800 cc. of a hæmorrhagic exudate in which there were large masses of clot. Both surfaces of the pericardium were covered with thick membranous masses containing hæmorrhages. A few gonococci were found in the pericardium. In Winterberg's case it is noted that 20 ccm. of sero-purulent fluid were found in the pericardial sac. In our second case the pericardial cavity contained about 300 ccm. of turbid yellow fluid containing flakes of fibrin. In the dependent portions the fluid was thick and puriform. The surface of the peri- and epicardium was congested and contained small ecchymoses; it was covered by a granular deposit. Gonococci were obtained in pure cultures from the fibrin upon the surface of the pericardium. In Rendu's case it is noted that the pericardium contained 500 cc. of translucent fluid and that there were "pericardial lesions," nothing further. Cultures from the fluid were negative.

Thus, it may be seen that anatomically nothing striking is to be made out from a consideration of these observations. The definite proof of the existence of a pure gonorrhœal pericarditis is furnished by the positive result of our cultures in Case II.

Myocarditis.—Grave myocardial changes, necroses with hæmorrhage, leucocytic infiltration, embolic abscesses have been described in a number of instances in association with fatal endocarditis. In Councilman's case the areas of necrosis and suppuration were large and gonococci were found microscopically in the foci.

CONCLUSIONS.

(1) An acute gonorrhœal urethritis may be the starting point for a grave general septicæmia with all its possible complications.

(2) These infections may be mixed or secondary, due to the entrance into the circulation of organisms other than the gonococcus, or they may be purely gonococcal in nature.

(3) Endocarditis is an occasional complication of gonorrhœa.

(4) This endocarditis may be transient, disappearing with but few apparent results, or it may leave the patient with a chronic valvular lesion, or it may pursue a rapidly fatal course with the symptoms of acute ulcerative endocarditis.

(5) The endocarditis associated with gonorrhœa is commonly due to the direct action of the gonococcus, but may be the result of a secondary or mixed infection.

(6) Pericarditis may also occur as a complication of gonorrhœa, but it is less frequent than endocarditis. It may, as in the case of the latter, be the result either of a pure gonococcal or of a mixed infection.

(7) Grave myocardial changes, necroses, purulent infiltration, embolic abscesses are common in the severe gonococcal septicæmias.

(8) In instances of gonococcal septicæmia the diagnosis may, in some cases, be made during life by cultures taken from the circulating blood according to proper methods.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(1) Bourdon. *Gaz. d. Hôp.*, 1868, xli, 1.	M	24	Gonorrhœa; one month later, arthritis; delirium; irregular chills; evidences of endocarditis. Hæmorrhagic areas in skin, becoming gangrenous; bed sores. Death 13 months after infection.	Verrucous endocarditis of mitral and tricuspid valves with thrombi. Kidneys pale and fatty.	
(2) Desnos. *L'Union Méd.* 3 s., 1878, xxv, 43. Also *Gaz. d. Hôp.*, 1877, L, 1867.	M	?	Gonorrhœa; bronchitis; arthritis; dyspnœa; palpitation; cardiac murmur; irregular fever. Death less than a month after onset of arthritis. The heart was normal on first examination. No history of any previous predisposing malady.	Vegetative endocarditis of aortic and mitral valves.	In the discussion of the case Fournier acknowledged its gonorrhœal origin.
(3) Schedler. "Zur Casuistik der Herzaffectionen nach Tripper." Inaug. diss. Berlin, 1880.	M	22	Gonorrhœa; epididymitis; arthritis and moderate fever, shortly after onset. 7 months later, irregularchills; evidences of aortic endocarditis with insufficiency. Death 5 weeks after onset of acute symptoms.	Ulcerative endocarditis of aortic valve.	Patient had had smallpox and a fever some time previously, which justifies one in asking whether the endocarditis may not have preexisted. At the autopsy the changes pointed to a recent affection. The reporter as well as Prof. Leyden have no doubt as to its gonorrhœal origin.
(4) Draper. *Medical Bulletin*, Philadelphia, 1882, iv, 81.	M	19	Chronic gonorrhœa for a year; arthritis; irregular fever, with chills; embolism in leg. Death 4 months after onset of arthritis.	Ulcerative endocarditis of mitral valve. Infarctions in spleen and kidneys.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(5) Martin. *Rev. Méd. de la Suisse Romande*, 1882, ii, 308, 352.	M	24	Previous history good. Lost appetite and began to feel ill 3–4 weeks before entrance into hospital. Chills; irregular fever; evidences of pyæmia; multiple arthritis; hæmaturia; right pleuro-pneumonia; suppurative conjunctivitis; parotitis. Death a month after beginning of symptoms.	Gonorrhœa; suppurative prostatitis; cystitis; suppurative inflammation of vesiculæ seminales; multiple myocardial abscesses. Ulcerative and vegetative endocarditis of mitral valve. In the thrombi on valves were numerous cocci, some of which were exactly similar morphologically to Neisser's gonococci. The kidneys showed fatty degeneration with septic emboli.	The case would appear to be of gonorrhœal origin, whether or not a secondary infection occurred.
(6) Fleury. *Journ. de Méd. de Bordeaux*, 1883-'84, xiii, 65.	M	27	Gonorrhœa; 3 months later disappearance of discharge; arthritis; thoracic pains; pericarditis. Death 3 weeks after arthritis. No signs of cardiac disease on examination before this infection.	Vegetative and ulcerative endocarditis of aortic valves with perforation. A certain amount of blood-stained fluid in the pericardium; a patch of false membrane just below the root of the aorta.	
(7) Weckerle. *Münch. med. Woch.*, 1886, xxxiii, 563, 582, 608, 622, 636.	F	21	Gonorrhœa; inguinal adenitis; arthritis; 1 to 2 months after arthritis, signs of ulcerative endocarditis of pulmonary valve; chills; irregular fever; dry pleurisy. Death a month after onset. The urine, normal at first, showed a large quantity of albumin; sediment; red and white corpuscles; renal epithelium and casts.	Sero-fibrinous pericarditis. Extensive ulcerative endocarditis of pulmonary valves. Numerous cocci in the thrombi on the valves, sometimes in chains, sometimes in groups. These organisms stain according to Gram. No particular search was made for gonococci. Kidneys large: subacute parenchymatous nephritis.	The author does not believe the endocarditis to have been gonococcal. The only portal of entry appears to have been furnished by the gonorrhœa.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(8) Weichselbaum *Centralbl. f. Bakt.*, 1887, ii, 209. Also, Ziegler's *Beiträge*, 1889, iv, 125.	M	21	Gonorrhœa for 3 weeks; high fever. Death on day of entry. Gonococci in urethral secretion.	Acute splenic tumor., Ulcerative aortic endocarditis. Gonococci in urethra. Only streptococci, morphologically and by culture, on the valves.	W. believes that the gonorrhœa formed the portal of entry for the streptococcus.
(9) Rothmund. "Endocarditis Ulcerosa." Dissertation, Zürich. 1889.	M	51	Gonorrhœa; 8 or 9 weeks later epididymitis; poly-arthritis; heart sounds clear; moderate fever. 3 weeks later loud systolic murmur over mitral and tricuspid areas. 10 days later, high pitched diastolic murmur over aortic and pulmonary regions; delirium; jaundice. Death 5 weeks after arthritis.	Urethritis; cystitis; right knee joint and left ankle contain sero-hæmorrhagic fluid. Extensive ulcerative endocarditis of aortic valves. In the blood of the right heart and in the affected joints were found cocci having the form and grouping of gonococci.	
(10) Ely. *Med. Record*, 1889, xxxv, 287.	M	28	No history. Ill 3 days. Fever; delirium; vomiting. 2 days after entry partial left hemiplegia and death.	Gonorrhœa; old pericardial adhesions; fresh vegetative and ulcerative endocarditis of mitral valve. In thrombi on valve there were cocci and bacilli. In all embolic abscesses in liver, kidneys and in heart valves as well as in urethra, similar cocci staining by Gram and arranged in clusters and chains, are seen. In urethral pus, gonococci decolorizing by Gram are also seen.	The author believes the case to be one of secondary infection with pyogenic cocci.

REPORTER.	SEX.	AGE	HISTORY.	AUTOPSY.	REMARKS.
(11) His. *Berl. klin. Woch.*, 1892, xxix, 993.	M	19	Gonorrhœa; first attack, disappearing apparently in 3 weeks. 2 weeks later syncopal attack, followed by chills; hæmorrhagic eruption; remittent fever; loud systolic murmur; signs of ulcerative andocarditis. Blood cultures from ear negative.	General anæmia; ecchymoses of skin and serous membranes; ulcerative aortic endocarditis; softened thrombus at cardiac apex. Acute interstitial myocarditis; septic emboli in spleen, kidneys and lungs. In the thrombi on the affected valve cocci resembling gonococci were found; these decolorized when treated according to Gram. Unfortunately, the specimen had been previously put in Müller's fluid.	
(12) His. *Op. cit.*	M	19	Gonorrhœa; arthritis; a few days later irregular fever and chills, sometimes two a day. 4 months after onset of gonorrhœa there were signs of ulcerative endocarditis; aortic stenosis. Death 5½ months after onset of gonorrhœa.	Acute aortic endocarditis; partial aneurism at root of aorta with papillary thrombi; enlarged spleen with fresh infarcts; subacute parenchymatous nephritis; slight hydrothorax; hydropericardium. No bacteriological examination.	Case observed by Wagner in 1879. Autopsy by Huber.
(13) Leyden. *Deutsch. med. Woch.*, 1893, xix, 909.	M	22	Chronic gonorrhœa; epididymitis; arthritis of right knee; signs of ulcerative endocarditis with aortic insufficiency; irregular fever; chills. Death 6 to 7 weeks after onset of arthritis. Cultures from vein on ordinary media, negative.	Acute myocarditis; ulcerative endocarditis of aortic and vegetative endocarditis of mitral valves. Typical gonococci decolorizing by Gram in the thrombi on valves. Cultures on ordinary media negative.	Doubtless a pure gonorrhœal endocarditis.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(14) Wilms. *Münch. med. Woch.*, 1893, xl, 745.	M	26	Gonorrhœa 6 months ago and again later; 3 weeks after second attack, chill, arthritis in knee; a week later signs of aortic insufficiency; high fever. Death 6 weeks after the second infection.	Ulcerative endocarditis of aortic valve extending through into right auricle; suppurative myocarditis. Numerous cocci in thrombus, some separate, some arranged like gonococci and intracellular. They decolorize almost immediately by Gram. In leucocytes in the sub-mucous tissue of urethra there were scanty diplococci, some resembling gonococci.	The author does not believe the organisms were gonococci though the description is more than suggestive.
(15) Golz. "Ulceröse Endocarditis der Klappen der Pulmonalarterie bei gonorrhoischer Arthritis." Inaug. Diss. Berlin, 1893.	M	21	Gonorrhœa; right-sided bubo; 6 days later arthritis in right shoulder and left foot. Gonococci found in urethra. 2 weeks later chills; heart sounds clear; chills and fever continued, and 2 weeks later systolic murmur became audible, diastolic murmur in pulmonary area. 3 weeks later pericarditis and pleurisy with hæmorrhagic exudate; pulmonary embolism. Death 3½ months after infection and a little less than 3 months after apparent onset of endocarditis.	Fibrinous pericarditis; in pericardium ½ litre of fairly clear fluid. Ulcerative endocarditis of pulmonary valve and of wall of right auricle. No cultures. The specimen had been left for a considerable time in alcohol before a bacteriological examination was made. Small cocci at times in groups and sometimes in chains were found. No characteristic gonococci.	The organisms described by the author appear to have been staphylococci and streptococci.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(16) Councilman. *Am. Jour. Med. Sci.*, 1893, cvi, 540.	M	?	Gonorrhœa; 10 days later arthritis in various joints. Five weeks after infection pericarditis. Death suddenly 3 days later.	Pericardium contained 800 cc. of hæmorrhagic exudate in which there were large masses of clot. Both surfaces of pericardium covered with thick membranous masses, containing hæmorrhages. Suppurative myocarditis. Gonococci, characteristic morphologically, in suppurative foci in heart muscle, pericardium, urethra and joints; they decolorized by Gram. No cultures.	
(17) Fressel. Inaug. Diss. Leipsic, 1894.	F	26	History imperfect. Severe symptoms came on a few days before death. Extreme weakness; emaciation; orthopnœa; arthritis of left ankle.	Ulcerative and polypoid endocarditis of mitral and aortic valves. Kidneys practically normal. Cystitis; urethritis; vaginitis. The thrombi on valves showed organisms having microscopically the characteristics of the gonococcus; some occupying cells; they decolorized by Gram's method. No cultures.	
(18) Winterberg. Festschrift z. 25 Jähr. Jub. des Vereins Deutscher Aerzte zu San Francisco, 1894, 10.	M	25	Gonorrhœa; 6 weeks later right epididymitis; double bubo; arthritis of both elbows; dyspnœa; systolic and diastolic murmurs especially in aortic area. Death after onset.	Pleural effusion on both sides; 20 cc. sero-purulent fluid in pericardium; myocardial abscesses. Ulcerative endocarditis of aortic and pulmonary valves which were almost entirely destroyed. Endocarditis of mitral and tricuspid valves of a lesser extent. Amyloid kidneys. No cultures. Cover glass specimens from valves showed organisms answering morphologically and tinctorially to gonococci, decolorizing by the method of Gram.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(19) Thayer and Blumer. *Arch. de Méd. Expér.*, 1895, vii, 701, and Johns Hopkins Hospital Bull., 1896, vii, 57.	F	34	Vague history of rheumatism 3 months ago; has been short of breath 3 or 4 years. About 3 months after "rheumatism," irregular fever and chills; signs of ulcerative endocarditis; mitral stenosis. Death 3 weeks after onset of fever. Cultures from median basilic vein 9 and 4 days before death showed pure growths of diplococci resembling in every way gonococci and decolorizing by Gram's method. The medium contained at least one-third blood (the syringe full of blood mixed with agar agar and plated). Transplanted to ordinary media there was no growth.	Ulcerative endocarditis of mitral valve. In the thrombi on valves and in vagina and uterus characteristic intracellular gonococci, decolorizing when treated according to Gram's method. Cultures made on agar agar and bullock's blood serum from all sources were negative.	This is the first case in which gonococci were obtained in pure culture from the blood during life.
(20) Finger, Ghon and Schlagenhaufer. *Arch. f. Dermat. u. Syph.*, 1895, xxxiii, 141, 323.	M	19	Chronic gonorrhœa for a year. Fresh attack in March, 1895. 6 months later arthritis in right knee; fever. Heart sounds clear; gonococci in urethral discharge; chills; 16 days later diastolic murmur over aorta, Death 9 days later.	Myocarditis; ulcerative aortic endocarditis with perforation. Arthritis of right knee joint. Chronic urethritis; prostatic abscess; infarct of spleen; cloudy swelling of kidneys. Characteristic gonococci in urethra and in thrombi on valves, decolorizing by Gram. Cultures on ox's serum peptone agar were negative excepting from urethra, from which an unidentified coccus was obtained.	The authors believe the case to be purely gonorrhœal, the gonococci having lost their vitality, possibly owing to high temperature before (and after, W. S. T.,) death, and hence failing to grow.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(21) Zawadzki and Bregman. *Wien. med. Woch.*, 1896, xlvi. 313, 351.	F	17	A month ago purulent vaginitis with staphylococcus and micrococcus tetragenus; chills and fever; 6 weeks after infection, arthritis in hip not yielding to salicylates; 11 days later, serous pleurisy on right side, showing on culture, streptococcus pyogenes; staphylococcus albus; micrococcus tetragenus. 15 weeks after onset right hemiplegia and death.	Verrucous mitral endocarditis; embolism of right arteria fossæ Sylvii. In the excrescences on valves among other organisms were numerous characteristic gonococci. These were in part in groups in the intermediate tissue, in part in the cells and in their neighborhood. They had the character and shape of gonococci and were decolorized when treated according to Gram. They were more numerous on the free border of the valves as were the other cocci.	A mixed infection. Some question as to whether the organisms found were gonococci.
(22) Dauber and Borst. *Deutsch. Arch. f. kl. Med.*, 1896, lvi, 231.	M	20	Gonorrhœa; tenosynovitis of left hand; inguinal buboes; periurethral abscesses. 2 weeks after onset, chill; irregular fever; heart's sounds clear. Pain in cervical vertebræ. 3 months after infection evidences of aortic insufficiency; septic nephritis. Gonococci gradually disappeared from urethral discharge, other bacteria appearing. Cultures from blood, negative. Death.	Ulcerative and vegetative endocarditis of aortic valve; suppurative myocarditis; acute nephritis; septic emboli and infarctions of kidney. In thrombi organisms showing all the characteristics of gonococci, decolorizing by Gram; also once in colorless corpuscle in heart's blood. Cultures negative excepting one on human blood serum agar on which there developed after 36 hours separate point-like, yellowish brown translucent colonies. On feeble magnification these appeared round, had a sharp clean-cut border, showed no outgrowths from their peripheries or daughter colonies so characteristic of gonococci. Micro-organisms obtained from the culture were almost entirely diplococci, in part biscuit shaped, in part, round.	While the authors doubt that these were gonococci, most will probably accept the case as positive. Vide Michaelis, *Op. cit.* and Thayer and Blumer. *Johns Hopkins Hospital Bulletin*, 1896, vii, 57.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(23) Michaelis. *Zeitschr. f. kl. Med.*, 1896, xxix, 556.	M	25	Gonorrhœa; 3 weeks later, arthritis; slight fever; no albuminuria; 4 days later, systolic murmur over aorta; diastolic sound not clear. Sudden death 10 days later. Gonococci in urethral discharge.	Vegetative and ulcerative aortic endocarditis with perforation. Pericardial and pleural cavities showed abundant serous fluid with a few fibrinous flakes. Purulent cystitis. Characteristic gonococci in thrombi on valves, i. e. shape; intracellular arrangement; decolorization by Gram's method; failure to grow on attempts to cultivate on ordinary media.	In all probability a pure gonococcal endocarditis.
(24) Keller. *Deutsch. Arch. f. kl. Med.*, 1896, lvii, 387.	M	25	Gonorrhœa; arthritis 4 weeks after; later pains in chest; chills; irregular intermittent fever; evidences of pulmonary endocarditis; pericarditis. Death 4 months after infection and 3 months after arthritis. Diagnosis: pulmonary stenosis and insufficiency.	Increase in pericardial fluid which was made cloudy by the presence of fine flocculi. Vegetative endocarditis of pulmonary valves and pulmonary artery; myocarditis. Streptococci in cultures from pericardial fluid. In polypi on pulmonary valve streptococci were found; streptococci in kidneys.	The author believes that this was a mixed infection through the urethra.
(25) Hale White. *Lancet*, 1896, i, 533.	M	19	Gonorrhœa; 3 weeks later, chills; irregular intermittent fever; anæmia; systolic and diastolic murmurs in pulmonary area; 5 weeks after infection, acute nephritis; œdema. Two weeks later, death.	Ulcerative and vegetative endocarditis of the pulmonary valve and artery. Acute nephritis; characteristic gonococci in vegetations on valves. These decolorized when treated by Gram's method. Cultures taken on agar, glycerine agar, broth and blood serum were without result. No cultures were made on serum agar. (These latter particulars were obtained in a personal communication from Dr. Pakes.)	The author remarks on the frequency of nephritis in ulcerative endocarditis, and believes it to be a not uncommon cause of death.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(26) Babes and Sion. *Arch. d. Sc. Méd. de Bucharest*, 1896, i, 505.	M	?	August 5, gonorrhœa; 20 days later cystitis; epididymitis; purpura; fever; chills; vomiting; splenic tumor. On September 2, aortic systolic murmur; albuminuria; diarrhœa. Death October 14. In blood during life Opresco saw cocci resembling gonococci and decolorizing by Gram; they were within leucocytes.	Gangrene of skin over lower abdomen and genitalia; hæmorrhagic infarct of kidney; ulcerative aortic endocarditis. In the thrombi on valves organisms similar to gonococci were found, decolorizing by Gram. On ordinary media saprophytes alone grew. No growths on beef blood serum agar. In spleen and kidney strepto- and staphylococci.	They believe the case to have been gonorrhœal with a secondary streptococcus and staphylococcus infection. They seem to think that, the pus cocci having entered and caused a general septicæmia, the gonococci profiting by the diminished resistance of the organism entered later and attacked the valves.
(27) Stengel. *Univ. Med. Mag.*, Phila., 1897, ix, 426.	F	20	Had had valvular heart disease, since rheumatism, at age of 7; gonorrhœa for a year (?); 6 days before entry into hospital diarrhœa; headache, vomiting, tympanites; evidences of mitral stenosis; acute nephritis. Continued fever. Death after 32 days.	Chronic endocarditis of mitral valve. Fresh ulcerative and vegetative endocarditis of mitral valve and part of auricle, particularly marked on an anomalous adventitious leaflet. Muco-purulent exudate in uterus and vagina. Cultures: Streptococcus from lungs and staphylococcus from right auricle and spleen; endocardial vegetations negative. Characteristic gonococci were found in two thrombi on valves, mainly intracellular, decolorizing by Gram. No gonococci found in exudate in uterus or vagina. Deeper tissues not examined.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(28) Thayer and Lazear. Subject of present communication, read before XII International Congress in Moscow, 1897. *Med. Record*, 1897, lii, 497.	M	19	Gonorrhœa 6 months before entry into hospital; several weeks later chill; 5 to 6 months later œdema of legs; subacute nephritis; grave anæmia; systolic murmur; pericarditis. Death 7½ months after infection, about 6 months after onset of chills. Gonococci obtained three times during life from circulating blood. Diagnosis: gonorrhœal septicæmia, endo- and pericarditis.	Ulcerative and vegetative endocarditis of tricuspid valve. Sero-purulent pleurisy and pericarditis; subacute hæmorrhagic and glomerular nephritis. Gonococci obtained microscopically and in pure culture on Loeffler's blood serum agar from heart's blood, pericardium and affected valves. All other cultures negative.	This is the first case in which gonococci were obtained in pure culture before and after death from the blood, thus permitting a positive ante-mortem diagnosis of gonorrhœal septicæmia.
(29) Rendu and Hallé. *Bull et. mém. Soc. méd. de hôp. de Par.* 1897, 3 s., xiv, 1325.	F	30	Gonorrhœal metritis; about 2 weeks later fever; night sweats; evidences of septicæmia. Gonococcus isolated from uterine mucus. Phlegmon near elbow joint; intermittent fever; endo- and pericarditis about 5 weeks after onset of fever. Death 10 days later. In phlegmonous œdema at elbow gonococci "à l'état de pureté" were found. Blood cultures negative.	Vegetative endocarditis of aortic valve and of ascending aorta. Sero-fibrinous pericarditis. Cultures from pleural and pericardial fluids negative. The bacteriological and histological examination of the aortic vegetations showed the exclusive presence of gonococci.	Were the gonococci obtained in pure culture?
(30) Siegheim. *Ztschr. f. kl. Med.* 1898, xxxiv, 526.	F	20	Chills and fever and systolic murmur in tricuspid area in June; later, systolic murmur in mitral, and diastolic in aortic area; dyspnœa; irregular intermittent fever; chills. Death July 11. Cultures from blood on Kiefer's agar agar and peptone bouillon were negative.	Ulcerative endocarditis of aortic valve; myocarditis; nephritis; purulent endometritis and cystitis. Cultures on Kiefer's agar from heart's blood and vegetations negative. Microscopical examination of smear preparations from thrombi revealed organisms showing all the morphological and tinctorial characteristics of gonococci.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(31) Lenharz. *Berl. kl. Woch.*, 1897, xxxiv, 1138.	F	19	Abundant vaginal discharge; symptoms of ulcerative endocarditis of pulmonary valve. Death.	Vegetative endocarditis of pulmonary valves. Characteristic gonococci in softened thrombi. These were obtained in pure culture. A piece of softened thrombus was introduced into the human urethra resulting in the development, after four days, of gonorrhœa with typical organisms.	This case would appear to remove all doubt as to the possibility of the existence of a true gonorrhœal endocarditis.
(32) Unpublished observation from the wards of Prof. Osler, Johns Hopkins Hospital. (Case observed by one of the authors—Thayer).	M	38	Measles as a child; no other acute infectious diseases. Entered hospital 20/vii/94. Has gonorrhœa with characteristic gonococci in discharge. For 3 weeks irregularly intermittent fever with chills. *Physical examination:* negative; heart sounds clear; apex impulse in 5th interspace, just inside mammillary line. Leucocytes 17,000 per cu. mm.; *urine* shows trace of albumin; no casts found. Irregular fever with severe rigors; left hospital unimproved on 2 / viii / 94. Urethral discharge and rigors stopped two weeks later; fever however continued. A few days after leaving hospital, stabbing pains in præcordial region, somewhat relieved by pressure over heart; dyspnœa. In latter part of October puffiness of eyelids, frequency of	(By Dr. Flexner.) Chronic and acute endocarditis (vegetative and ulcerative) of the pulmonary valves; deficiency of one segment; vegetation extending 3 cm. into the pulmonary artery; globular thrombi in left ventricle; acute pneumonia; chronic diffuse nephritis. Coverslips from the thrombi on the affected valve show encapsulated diplococci; others biscuit shaped, resembling gonococci. On treatment by Gram's method some apparently decolorized, others did not. Characteristic gonococci in urethra. Cultures on ascitic fluid agar and Loeffler's blood serum from thrombi on affected valve, mitral valve, heart's blood, pleura, lung, kidney, spleen, liver and bladder all showed pneumococci.	The clinical history suggests strongly that the original process was associated with the gonorrhœa, while the pneumococcus infection was a late secondary event. The following note was made by Dr. Flexner: "Note.— Had the gonococcus been present it is probable that it would have been so obscured by the universal presence of the the Diploc. pneumoniæ that it could not have been detected."

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
			micturition, purpuric eruption on legs. Re-entered hospital 7/xi.—Physical examination: puffiness of face; pulse of high tension. Heart: apex impulse in 5th space 2 cm. outside nipple; slight to-and-fro murmur over pulmonary orifice, so superficial that it was believed to be pericardial; purpuric eruption on legs and thighs. *Urine:* trace of albumin; sediment: hyaline, granular, pus casts and renal epithelial cells. *Blood:* 13/xi; red corpuscles, 3,920,000; colorless, 31,200; hæmoglobin, 46%. 14/xi; diastolic murmur, heard by Prof. Osler in pulmonary area. Irregular fever disappeared on the 18th and T° was subnormal afterwards, excepting on the 21st, 22nd and 23rd. The dyspnœa increased, pneumonia developed, and on 26 / xi the patient died. The day before death the leucocytes were 78,000 per cu. mm. Cultures from the blood, made by Dr. Blumer on 17/xi, were negative.		

ON ALTERATIONS IN THE COMPOSITION OF THE BLOOD RESULTING FROM EXPERIMENTAL DOUBLE NEPHRECTOMY.

By C. A. HERTER, M. D., and A. J. WAKEMAN, Ph. D.

Although the alterations in the composition of the blood which follow uncomplicated experimental double nephrectomy are of much interest in relation to the pathology of human uræmic states, the literature relating to the subject is exceedingly limited, and it is certain that investigators have not given it the attention of which it is worthy. It is the aim of this paper to present concisely the results of experimental work relating chiefly to the chemical changes that occur in the blood as the result of double nephrectomy. In most instances dogs were employed as the subjects of these observations, and both kidneys were removed at one operation. The chief symptoms following the removal of both kidneys in dogs are as follows: lowering of the temperature, loss of appetite, drowsiness, vomiting, diarrhœa.

In a few instances the second kidney was removed several days after the removal of the first. In a smaller series of cases studies were made of the blood after both ureters had been tied just below the pelves of the kidneys. In almost all cases the animals were carefully watched, and were bled to death as soon as there were indications that life was not likely to be prolonged for many hours. This method of procedure is of importance, because there is reason to think that the composition of the blood undergoes alterations in certain respects by contact post mortem with the tissues.*

Thus v. Limbeck obtained the following results:

	NaCl.		KCl.		HNa_2PO_4	
	During life.	48 hours post mort.	During life.	48 hours post mort.	During life.	48 hours post mort.
Blood..........	.1643	.1642	.0279	.0567	.0247	.0327
Muscle.........	.0193	0.198	.3261	.2271	.3286	.2721

* v. Limbeck, *Arch. f. exp. Path. u. Pharm.*, 1892, xxx, 195.

Our observations have reference to the reaction of the blood and to its content of urea, of uric acid, of alcoholic and ethereal extractive substances, of ethereal extractive substances, of total proteids, of fibrin, of ash, of sodium and potassium, and of phosphoric acid.

*Reaction of the blood.**—Reference to Table I shows that the alkalinity of the blood was distinctly increased as the result of double nephrectomy. Thus the average alkalinity in six normal dogs was .0043 grm. NaOH (1 cc. blood), while that of six nephrectomized dogs was .0095 grm. NaOH. In the case of two dogs whose ureters had been tied, the alkalinity gave an average of .0138 grm. NaOH, but these results do not of course justify the conclusion that a higher grade of alkalinity is to be regularly expected after ligation of the ureters than after nephrectomy.

TABLE I.—ALKALINITY OF BLOOD.

No.	Nature of experiment.	No. of hours.	1 cc. blood in terms of cc. of $\frac{1}{10}$ normal tartaric acid sol.	1 cc. blood in terms of grammes of NaOH.	NORMAL DOG'S BLOOD. 1 cc. blood in terms of cc. of $\frac{1}{10}$ normal tartaric acid sol.	NORMAL DOG'S BLOOD. 1 cc. blood in terms of grammes of NaOH.
	Double					
1	Nephrectomy.	48	4.10 cc.	.0164 grms.	.940	.0037 grms.
2	"	52	1.65 "	.0066 "	.950	.0038 "
3	"	52	2.91 "	.0116 "	.949	.0037 "
4	"	52	2.21 "	.0088 "	.730	.0029 "
5	"	72	1.44 "	.0057 "	1.05	.0042 "
6	"	72	1.93 "	.0077 "	1.90	.0076 "
	Both ureters					
7	ligated.	48	3.10 "	.0124 "		
8	"	52	3.80 "	.0152 "		

Average of results, Nos. (1–6) = 2.37 cc. = .0095.
" " " " (7–8) = 3.45 cc. = .0138.
" " " " (1–8) = 2.64 cc. = .0106.
" " " " Normal = 1.08 cc. = .0043.

This increased alkalinity after nephrectomy is especially noteworthy, as it contrasts with the well-known results of von Jaksch in

* *Method:* To a given amount of freshly drawn blood a tenth normal solution of tartaric acid containing sodium sulphate is added. The point of neutralization is determined by the use of litmus paper. For the details of the process see Hoppe-Seyler's Handbuch d. physiol.- u. path.-chem. Analyse, p. 399, Berlin, 1893. The method for absolute values is far from accurate on account of the difficulties involved in determining the neutral point, but is probably sufficiently accurate to render relative results trustworthy.

human uræmia.† It is very doubtful if it can be ascribed entirely to loss of water, as the increase in the specific gravity of the blood is inconsiderable.

*Urea.**—The increase in the percentage of urea in the blood is perhaps the most striking alteration in the composition of the blood after double nephrectomy (Table II). Twenty-eight observations on the blood of normal dogs give an average percentage of .037. Eighteen observations on nephrectomized dogs (which lived from 22 to 82 hours) yield an average of .315 per cent, while six observations on the blood of dogs whose ureters were tied give an average of .301 per cent. The increase in the percentage of urea thus amounts to nearly 10 times the average normal percentage in the course of 82 hours or less. The highest individual percentage in the blood was .458, or nearly .5 per cent. A similar increase in the urea of the blood has been observed by us in several cases of uræmia. It is noteworthy that the average normal percentage of urea in human blood is about the same as that of dogs, and that the highest percentages of urea which have been found in human uræmia are comparable with the highest noted after nephrectomy.

The urea which is stored in the blood after removal of the kidneys is derived from the waste of proteid tissue, and perhaps in a measure directly from proteid food in the digestive tract at the time of opera-

† v. Jaksch. *Zeitschr. f. klin. Med.*, xiii, 350. Further work is certainly needed to determine whether the diminished alkalescence of the blood observed by von Jaksch is actually a feature of the blood in human uræmia.

* *Method:* A given quantity of blood is treated with four or five times its volume of absolute alcohol and allowed to stand 24 hours. The filtrate and washings are evaporated to dryness at a moderate temperature, and the residue is taken up in absolute alcohol and filtered. The filtrate and washings are again evaporated to dryness, and the residue dissolved in a little water. This solution is subjected to the action of sodium hypobromite, and from the volume of nitrogen evolved is calculated the percentage of urea. Other nitrogenous alcoholic extractives of blood, as creatin and lecithin, are partly decomposed with liberation of nitrogen in this process, and herein lies the inaccuracy of the method. Urea, however, is by far the most abundant of the nitrogenous extractives, and the only one which is wholly broken up by the action of sodium hypobromite. The results obtained by this method are looked upon as sufficiently accurate for the purposes of this investigation.

tion.* After operation the animals seldom eat, and the formation of urea is probably little greater than in a state of starvation. The waste in starvation is, however, considerable. A dog weighing 12 kilos, and possessing, say, one kilo of blood, would produce enough urea to cause an accumulation of .5 per cent of urea in the blood in the course of three days, supposing the formation of urea to be even less than 2 grammes daily and further supposing the blood to be entirely free from urea at the time of operation. The daily formation of urea in a starving dog weighing 12 kilos is, however, considerably greater than 2 grammes daily. If all the urea formed were stored in the blood, the percentage would probably rise to 1 in three days. The discrepancy between the amount which should theoretically be found and the amount actually stored in the blood can probably be explained wholly by the fact that after nephrectomy urea

TABLE II.—Urea in the Blood.

No.	Nature of experiment.	No. of hours.	Percentage of urea.	Normal dog's blood. Percentage of urea.	No.	Nature of experiment	No. of hours.	Percentage of urea.	Normal dog's blood Percentage of urea.
1	Double nephrectomy	22	.394	.049	19	Both ureters ligated	48	.260	.027
2	"	26	.256	.040	20	"	36	.342	.058
3	"	36	.103	.035	21	"	45	.200	.021
4	"	40	.121	.023	22	"	48	.323	.063
5	"	48	.350	.023	23	"	52	.302	.022
6	"	48	.395	.026	24	"	72	.377	.011
7	"	48	.276	.021	25	Left kidney removed, right ureter ligated.	24	.254	.013
8	"	48	.184	.034					
9	"	48	.347	.070					
10	"	52	.259	.036					
11	"	52	.366	.079	26	Right ureter ligated, left renal ligated.	36	.247	.036
12	"	52	.286	.073					
13	"	60	.431	.042					
14	"	60	.386	.025					
15	"	72	.458	.031	27	Both renals ligated.	36	.330	.027
16	"	72	.456	.045					
17	"	72	.220	.027	28	Both renals ligated, both ureters ligated.	36	.310	.027
18	"	82	.377	.049					

Average of results: Nos. (1–18) = .315.
" " " (19–24) = .301.
" " " (1–28) = .308.
" " Normal = .037.

* Chittenden. On Digestive Proteolysis, p. 105, New Haven, 1895.

accumulates in the muscles, brain, and other tissues and organs. A similar storage in the tissues is noticeable both in human uræmia and after the experimental intravenous injection of a solution of pure urea in dogs, and makes the percentage of urea in the blood lower than it would be were all the urea retained in the blood.

*Uric acid.**—The few observations upon uric acid recorded in Table III indicate that the salts of uric acid are not increased after nephrectomy—the observations, however, are too few to be conclusive.

If it be a fact that uric acid occurs in minute quantities in normal blood and that it is not increased after nephrectomy, this is of considerable physiological interest. Speculations on the possible reasons for the absence of an increase are of little interest until the fact itself is established.

TABLE III.—Uric Acid in the Blood.

No.	Nature of experiment.	No. of hours.	Percentage of uric acid.	Remarks.	Normal dog's blood. Percentage of uric acid.	Remarks.
1	Double nephrectomy.	48	.0016	Residue did not respond to murexide test.	.0022	Residue did not respond to murexide test.
2	Both ureters ligated.	48	.0025	" "	.0014	" "
3	" " "	52	.0024	" "		

Average of results: Nos. (1 to 3) = .0022.
" " " Normal = .0018.

Alcoholic and ethereal extractives.†—A moderate increase in the alcoholic and ethereal extractives appears to be a feature of the blood after removal of both kidneys. Thus the average of fourteen determinations on the blood of nephrectomized dogs gives 1.29 per cent, while the average of thirteen results obtained from the study of

* *Method:* After the removal of the proteids in the well-diluted blood by heat and acetic acid, the method employed was essentially that of Salkowski modified by von Schroeder. See Hoppe-Seyler's Handbuch d. physiol.- u. path.-chem. Analyse, p. 398, Berlin, 1893.

† *Method:* A given quantity of blood is treated with a large excess of alcohol and allowed to stand. The precipitate is washed with hot absolute alcohol, and afterwards extracted with ether. The alcoholic filtrate and washings are evaporated and the residue extracted and washed with cold and hot absolute alcohol. The combined alcoholic and ethereal residues are dried at 105° C. and the ash deducted.

normal dog's blood gives .95 per cent (Table IV). A similar moderate increase is often observed in the blood of fully developed human uræmia, and also in the blood in some infectious conditions which do not give the typical clinical evidences of uræmia. The alcoholic and ethereal extractives include in larger or smaller quantities fats, cholesterin, lecithin, creatin, urea and salts of hippuric acid, sarco-lactic acid and carbamic acid, sugar and xanthin bodies.

TABLE IV.—ALCOHOLIC AND ETHEREAL EXTRACTIVES OF THE BLOOD.

No.	Nature of experiment.	No. of hours.	Percentage alcoholic and ethereal extractives.	Normal dog's blood. Percent. alc. and eth. extractives.
1	Double nephrectomy.	36	1.02	1.11
2	" "	40	1.33	1.10
3	" "	48	1.13	1.33
4	" "	48	1.41	1.03
5	" "	48	.97	.88
6	" "	48	1.13	.83
7	" "	48	1.31	.98
8	" "	52	1.26	.85
9	" "	52	1.54	.82
10	" "	52	1.00	.88
11	" "	60	1.72	.80
12	" "	72	1.13	.80
13	" "	72	1.31	.94
14	" "	82	1.76	
15	Both renals ligated.	36	1.76	
16	Both ureters ligated.	45	1.10	

Average of results: Nos. (1–14) = 1.29.
" " " (1–16) = 1.30.
" " Normal. = .95.

*Ethereal extractives.**—The percentage of increase in the ethereal extractives is greater than that of the combined alcoholic and ethereal extract. It is seen in Table V that the average of eight results obtained from the blood of dogs whose kidneys had been removed or ureters tied is .299, which is more than double the average result obtained from the study of the blood of six normal dogs. The

* *Method:* A known weight of blood is dried and a given quantity of the finely powdered residue is thoroughly extracted in Soxhlet's extraction apparatus. The extract is dried at 105° C. and weighed and the ash deducted.

ethereal extractives of blood include neutral fats and cholesterin, as well as lecithin and salts of hippuric acid, and possibly of sarcolactic and carbamic acids.

TABLE V.—Ethereal Extractives of the Blood.

No.	Nature of experiment.	No. of Hours.	Percentage of ethereal extractives.	Normal dog's blood. Percentage of ethereal extractives.
1	Double neprectomy.	40	.378	.206
2	" "	48	.308	.104
3	" "	52	.295	.144
4	" "	52	.320	.180
5	" "	72	.358	090
6	" "	72	.130	.100
7	Both ureters ligated.	48	.270	
8	" " "	52	.330	

Average of results: Nos. (1-6) = .298.
" " " " (1-8) = .299.
" " " Normal = .137.

*Total proteids.**—The total proteids of the blood in six normal dogs yielded an average of 6.95 per cent of the constituents of the blood (Table VI). The average percentage of the total proteids in seven

TABLE VI.—Total Proteids of the Blood.

No.	Nature of experiment	No. of hours.	Percentage of total proteids.	Normal dog's blood Percentage of total proteids.
1	Double nephrectomy.	26	6.49	7.07
2	" "	48	9.54	7.44
3	" "	52	6.61	6.90
4	" "	52	8.82	5.10
5	" "	52	7.40	7.11
6	" "	72	7.38	8.09
7	" "	72	7.39	
8	Both ureters ligated.	45	6.55	
9	" " "	48	8.19	
10	" " "	52	6.40	

Average of results: Nos. (1- 7) = 7.66.
" " " (8-10) = 7.05.
" " " (1-10) = 7.48.
" " Normal = 6.95.

* *Method:* The proteids are precipitated in large excess of alcohol. After standing they are brought upon a weighed filter paper, washed with hot alcohol and ether, dried, weighed and the ash deducted.

cases of double nephrectomy was 7.66 per cent and 7.05 per cent in the case of three dogs in which double ligation of the ureters was practised. The inference seems proper that the percentage of the total proteids remains unaltered both after double nephrectomy and after double ligation of the ureters.

*Fibrin.**—The average fibrin yield of the blood of eleven normal dogs was .246 per cent. The average yield from seven nephrectomized dogs was .513 per cent. The average yield from three dogs in which the ureters had been tied was .483 per cent (Table VII). It is not possible at present to account satisfactorily for this apparently constant increase in the fibrin content of the blood after double nephrectomy. At first the possibility suggested itself that the increase might be the result of the simple elimination of the renal functions, but control observations have failed to establish this idea. In some of our operations there was moderate hemorrhage. It is known that considerable hemorrhage is followed by an increase in the fibrin content of the blood, and the question arose whether the increase of fibrin after nephrectomy might not depend on hemorrhage. Several operations were then done with great care to avoid hemorrhage. The fibrin content of the blood in these cases was as high as in the case of the dogs previously nephrectomized. As various infections are associated with an increase of fibrin, and as streptococcus infection occurred in some of our dogs, it seemed possible that our high fibrin results were due to infection. But this explanation would hold true of only a few of our cases; the fibrin content of the blood was increased even in cases where infection could be excluded. It was further found that if both hind legs of rabbits were removed at the knee, the fibrin of the blood was distinctly increased on the third day, even when hemorrhage and infection were absent. Thus from one experiment of this nature the fibrin yield was .702 per cent, and from another .744 per cent, while the average fibrin yield of the blood of eight normal rabbits was .255 per cent. On the other hand a simple

* *Method:* The fibrin estimations were made by the method of Hoppe-Seyler, see Handbuch, p. 410. The fibrin, separated by beating the freshly drawn blood, is thoroughly washed with a dilute solution of sodium chloride, then with water, and finally with boiling alcohol. It is dried at 105° C. and weighed. Duplicate analyses gave uniformly concordant results.

laparotomy on a dog was not followed by any change from the average fibrin content. On the whole it seems more likely that the increase in the fibrin of the blood after nephrectomy is in some obscure way connected with the operation to which the animal is subjected rather than to the elimination of the renal functions *per se*.

TABLE VII.—FIBRIN FROM THE BLOOD.

No.	Nature of experiment.	No. of hours.	Percentage of fibrin.	Normal dog's blood. Percentage of fibrin.
1	Double nephrectomy.	48	.457	.201
2	" "	48	.430	.162
3	" "	52	.585	.221
4	" "	52	.450	.375
5	" "	52	.491	.320
6	" "	72	.690	.320
7	" "	72	.490	.185
8	Both ureters ligated.	45	.600	.200
9	" " "	48	.530	.279
10	" " "	52	.320	.240
				.200

Average of results: Nos. (1– 7) = .513.
" " " (8–10) = .483.
" " " (1–10) = .504.
" " Normal = .246.

*Ash.**—By referring to Table VIII it is seen that the average of eight estimations of the ash from the blood of nephrectomized dogs

TABLE VIII.—ASH OF THE BLOOD.

No.	Nature of experiment.	No. of hours.	ercentage of ash	Normal dog's blood. Percentage of ash.
1	Double neprectomy.	40	.74	.93
2	" "	48	.91	.83
3	" "	48	.90	.94
4	" "	52	.96	.48
5	" "	52	.93	.92
6	" "	52	.72	.64
7	" "	72	.84	
8	" "	72	1.02	
9	Both ureters ligated.	45	1.09	
10	" " "	48	.99	

Average of results : Nos. (1-8) = .88.
" " " " (9-10) = 1.04.
" " " " (1-10) = .91.
" " " Normal = 79.

* *Method:* A given quantity of blood is dried and decarbonized with access of air over a Bunsen burner; the residue is weighed.

is .88 per cent, while the average of six estimations from the blood of normal dogs is .79 per cent. These results would seem to indicate an increase of the non-volatile products of the blood in the case of nephrectomized animals. The difference, however, is so slight, and the observations so few, that one is not justified in attaching much importance to the suggestion.

TABLE IX.—SODIUM AND POTASSIUM IN THE BLOOD.

No.	Nature of Experiment.	No. of Hours.	Percentage of Na_2O.	Normal dog's blood Percentage of Na_2O.	No.	Nature of experiment.	No. of hours.	Percentage of K_2O.	Normal dog's blood Percentage of K_2O.
1	Double nephrectomy.	36	.324	.280	1	Double neneprectomy.	36	.067	.015
2	" "	40	.156	.273	2	" "	40	.028	.026
3	" "	48	.213	.273	3	" "	48	.028	.020
4	" "	48	.345	.240	4	" "	48	.027	.036
5	" "	52	.330	.284	5	" "	48	028	.022
6	" "	52	.365		6	" "	48	.034	.015
7	" "	52	.260		7	" "	52	.021	.017
8	" "	60	.181		8	" "	52	.031	.029
9	" "	72	.305		9	" "	52	.033	.027
10	" "	72	.285		10	" "	60	.030	
11	Both ureters ligated.	45	.396		11	" "	72	.032	
12	" "	48	.392		12	" "	72	.035	
13	" "	52	.418		13	" "	72	.031	
					14	Both ureters ligated.	45	.029	
					15	" "	48	.036	
					16	" "	52	.038	
					17	" "	48	.027	

Average of results: Na_2O, Nos. (1-10) = .276.
" " " Na_2O, " (11-13) = .401.
" " " Na_2O, " (1-13) = .305.
" " " Normal, Na_2O = .270.
" " " K_2O, Nos. (1-13) = .033.
" " " K_2O, " (14-17) = .033.
" " " K_2O, " (1-17) = .033.
" " " Normal, K_2O .023.

*Sodium and potassium.**—The blood from ten nephrectomized dogs gave an average of .276 per cent Na_2O, and three dogs whose ureters had been tied yielded an average of .401 per cent Na_2O (Table IX). Estimations on the blood of five normal dogs showed

* *Method:* A given quantity of blood is carbonized in presence of $(NH_4)_2SO_4$. The residue is extracted with water, and a mixture of $BaCl_2$ and $Ba(OH)_2$ added. The barium is thrown out of the filtrate with $(NH_4)_2CO_3$. The method of procedure is as usual. The weight of the combined chlorides of sodium and potassium is obtained, and the potassium alone is weighed as K_2PtCl_6.

an average of .270 per cent Na_2O. It is thus seen that the sodium content of the blood of nephrectomized dogs is about the same as that found in normal animals. The percentage of sodium in the blood of animals whose ureters had been tied is seen to be considerably higher than the normal, but more observations are needed to determine whether this is regularly the case.

As regards the potassium content of the blood, there is found a slight increase in the case of dogs whose kidneys have been removed or ureters ligated. Thus the average yield from the study of the blood of thirteen nephrectomized dogs is found to be .033 per cent K_2O, and from four dogs whose ureters had been tied the value is the same. The blood of nine normal dogs yielded an average of .023 per cent K_2O.

TABLE X.—PHOSPHORIC ACID IN THE BLOOD.

No.	Nature of experiment.	No. of hours.	Percentage of P_2O_5.	Normal dog's blood. Percentage P_2O_5.
1	Double nephrectomy.	48	.100	.111
2	" "	52	.134	.068
3	" "	52	.144	.064
4	" "	72	.115	.063
5	" "	72	.145	
6	Both ureters ligated.	45	.066	
7	" " "	48	.081	
8	" " "	52	.077	

Average of results: Nos. (1–5) = .128.
" " " (6–8) = .075.
" " " (1–8) = .106.
" " Normal = .076.

*Phosphoric acid.**—The study of the blood of five nephrectomized dogs yielded an average of .128 per cent P_2O_5. From three dogs whose ureters had been tied an average of .075 per cent P_2O_5 was obtained. The average from the study of the blood of four normal dogs was found to be .076 per cent P_2O_5 (Table X). These results

* *Method:* A weighed amount of blood is decarbonized by aid of HNO_3. The phosphates in HNO_3 are precipitated with molybdic acid mixture. The precipitate is filtered off and washed and dissolved in $(NH_4)OH$. The phosphates are again precipitated with "Magnesia Mixture," and finally weighed as $Mg_2P_2O_7$.

suggest an increase of the salts of phosphoric acid in the blood of nephrectomized dogs—a suggestion borne out by the study of the potassium content of the blood, as well as the ash, before and after nephrectomy.

Muscle, liver and brain.—The results recorded in Table XI represent the percentage of nitrogen obtained by the action of sodium hypobromite in alkaline solution upon the alcoholic extractives of the various organs.*

In the case of the muscle it is seen from the average of a number of observations that the nitrogen evolved by the action of sodium hypobromite upon the alcoholic extractives after nephrectomy is four to five times as great as that obtained from the alcoholic extractives of normal dog's muscle. A still greater increase is noticeable in the case of the liver. Thus the alcoholic extractives of the liver after nephrectomy liberate about nine times as much nitrogen as the alcoholic extractive of the normal liver. The few observations on the brain show the same marked increase.

While no attempt was made to establish the identity of the substance or substances which contribute to the increased liberation of nitrogen after nephrectomy, it is fair to suppose that the increase was due to the accumulation of urea. This view is borne out by the following experiment. Into the femoral vein of a dog weighing 43 pounds, whose kidneys had been removed, 220 cc. of a 25 per cent urea solution were injected at the rate of 20 cc. per minute. The animal was bled to death immediately after injection and the muscle, freed from blood, gave .066 per cent N when the alcoholic extract was acted upon by sodium hypobromite. Muscle cut from the animal before injection gave .036 per cent N. The increase here is clearly due to urea, since the other muscle extractives during the short time involved would remain fairly constant.†

* The method of obtaining the alcoholic extractives preparatory to the action of sodium hypobromite was similar to that pursued in the estimation of urea in blood. (See foot-note, p. 119.)

† In another case where a similar experiment was made, but in which the rate of infusion was much slower (2 cc. per minute), the urea rose to .648 per cent in the muscles, to .808 per cent in the liver and to .593 per cent in the brain.

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TABLE XI.—Nitrogen, by Action of Sod. Hypobromite upon Alcoholic Extractives, of Muscle, Liver and Brain.

No.	Muscle. Nature of experiment.	Muscle. No. of hours.	Muscle. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.	Muscle. Normal dog's muscle. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.	Liver. Nature of experiment.	Liver. No. of hours.	Liver. Percentage of hydrogen by action of sod. hypobromite upon alcoholic extractives.	Liver. Normal dog's liver. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.	Brain. Nature of experiment.	Brain. No. of hours.	Brain. Percentage of nitrogen by action of sod. hopobromite upon alcoholic extractives.	Brain. Normal dog's brain. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.
1	Double nephrectomy.	48	.098	.028	Double nephrectomy.	48	.048	.0075	Double nephrectomy.	48	.125	.048
2	"	48	.087	.029	"	48	.104	.0098	"	60	.132	.030
3	"	48	.125	.019	"	48	.091	.0102	"	60	.138	
4	"	60	.125	.036	"	60	.107	......	R. ureter tied, L. renal tied.	36	.091	
5	"	60	.158	.035	"	82	.061	......	Both renals tied.	36	.053	
6	"	82	.230	.033	Both ureterstied.	36	.146					
7	Both ureters tied.	36	.152	.034	Both renals tied.	36	.047					
8	R. ureter tied, L. renal tied.	36	.084									
9	Both renals tied.	36	.020									

Average: Nos. (1–6) muscle = .137
" Normal " = .031

Average: Nos. (1–5) liver = .082
" Normal " = .0092

Average: Nos. (1–3) brain = .132
" Normal " = .039

The results from the organs of nephrectomized dogs are mainly interesting when compared with the results similarly obtained from the organs of normal dogs. It is doubtful whether the nitrogen derived from the normal organs, especially in the case of the muscle, is due to the presence of urea. Thus Nencki and Kowarski* by employing phosphotungstic acid as a precipitant in the aqueous extract of large quantities of dog's muscle, obtaining an alcoholic extract, and eventually applying a delicate color test by the use of orthonitrobenzaldehyde and phenylhydrazin, arrived at the conclusion that urea is not a normal constituent of the muscle.

Few, if any, of the nitrogenous extractives except urea are wholly decomposed with liberation of nitrogen by the action of alkaline hypobromites, and upon most of the nitrogenous extractives the action of sodium hypobromite with liberation of nitrogen is slight. This favors the view that the increased nitrogen after nephrectomy is due to urea.

* *Arch. f. exp. Path. u. Pharm.*, 1895, xxxvi, 395.

ON THE INFLUENCE OF FASTING UPON THE BACTERICIDAL ACTION OF THE BLOOD.

By S. J. MELTZER, M. D., and CHARLES NORRIS, M. D.

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In the following we shall report the results of some experiments which we have made to ascertain the influence of fasting upon the bactericidal action of the blood. We were led to this line of experimentation by the casual observation made in our investigation of the bactericidal action of the lymph from the thoracic duct of the dog,* that our parallel experiments with the blood did not show such an intense germicidal effect as most other writers on this subject had observed. It occurred to us that the weaker bactericidal action observed by us may have been due to the fasting of our animals. In order to compare satisfactorily the number of colonies in the different plates, we had to obtain lymph free from the misleading fine droplets of chyle, and, therefore, our dogs were allowed to fast for 30 to 40 hours previously to their being operated upon. The investigations of other writers have not had this motive, and we may presume that in their experiments the blood was obtained within a few hours after feeding the animals.

The assumption that inanition might reduce the bactericidal action of the blood seemed to us plausible for the following reasons: It is now generally conceded that the bactericidal powers of the blood are generated in some way by the leucocytes. The hyperleucocytosis of digestion is a well established fact, and on the other hand, Luciani† found in a fasting human being (Succi) and Rieder‡ in fasting dogs a marked hypoleucocytosis. It is therefore reasonable to assume that the bactericidal elements are increased also during digestion, and

* Meltzer and Norris, *Journal of Experimental Medicine*, 1897, ii, 701.

† Luciani, Das Hungern, Hamburg und Leipzig, 1890.

‡ Rieder, Beiträge zur Kenntniss der Leukocytose u. s. w. Leipzig, 1892.

decreased in a state of inanition. Furthermore, if we consider the bactericidal elements as the natural protectors of the body against infection our hypothesis would coincide with the generally acknowledged fact, that a poorly nourished body is more apt to be attacked by an infectious disease than a well-fed one, a fact which finds support in the experiments of Canalis and Morpurgo,* showing that starving pigeons lose their natural immunity from the infection with anthrax bacilli.

To test the validity of this hypothesis was the object of our experiments. We restricted our experiments to dogs and the typhoid bacillus. The blood was obtained under the usual precautions either from the femoral or the carotid arteries under cocaine anæsthesia. The comparisons were made between specimens of blood from the same dog and similar arteries under different conditions of feeding. Serum and blood defibrinated by shaking with glass beads were examined in each experiment. The bactericidal power was studied by the Buchner method, *i. e.* a quantity of blood or serum was inoculated with the typhoid bacillus: from this plates were made immediately after inoculation, and then one hour, two hours, etc., after inoculation other plates were made, and the number of colonies on the different plates counted. In some of the experiments two series of observations were made; one with serum kept at 37° C., the other with serum kept at room temperature. We were often compelled by outside reasons to keep the blood two days or longer in the ice-box before the bactericidal power could be tested, but then with few exceptions the blood of the well-fed and of the fasting animal were kept under exactly the same conditions.

Exp. B^1. Medium-sized male dog. Fed twice daily for two days. Nov. 4, a few hours after feeding, blood was taken from the right femoral artery. The defibrinated blood and the serum were kept in the ice-chest for 3 days, then inoculated and plated with the following result:

37° C.	Immediate.	1 hr.	6½ hrs.	25 hrs.	4 days.
Blood	5899	42	4	34916	....
Serum	1499	90	0	29	40000

* Canalis and Morpurgo, *Fortschr. d. Med.*, 1890, viii, 693 and 729.

Exp. B². The same dog, after fasting for five days, on Nov. 9 (previous wound having healed per priam) was operated upon and blood was taken from the left femoral artery. Blood and serum kept in ice-box for 20 hours, then inoculated and plated.

37° C.	Immediate.	1 hr.	3½ hrs.	25 hrs.
Blood	3445	1197	60	6169
Serum	2210	496	60	13992

In this experiment there seems to be a slight difference in favor of the blood from the well-fed animal, although it was kept longer in the ice-box than the blood from the fasting animal.

Exp. C¹. Small female dog fed a few hours before operation, Nov. 17; blood taken from the right femoral artery; blood and serum kept 40 hours in ice-chest, then inoculated and plated.

37° C.	Immediate.	1 hr.	3½ hrs.	6½ hrs.	24 hrs.
Blood	2316	169	9	4	27
Serum	689	79	19	15	194
21° C.					
Blood	1012	567	265	69	9
Serum	625	418	116	90	12

Exp. C². Nov. 22 from the same dog, the previous wound having healed, fasting (without water) since the first operation, blood was taken from left femoral artery; the blood and serum were kept 48 hours in ice-box, then inoculated and plated.

37° C.	Immediate.	1 hr.	3½ hrs.	6 hrs.	24 hrs.
Blood	4086	583	43	8	81
Serum	5633	1155	123	4	390
21° C.					
Blood	3079	1706	678	413	185
Serum	5194	2234	1303	483	68

In this experiment there is apparently no difference between both kinds of blood and serum. Six hours after inoculation at 37° C., there is about the same reduction in all the specimens of the blood and the serum, though there was a higher initial number of colonies in the blood and the serum taken from the starved animal. The fasting apparently did not reduce the bactericidal power of the blood.

Exp. D¹. Large dog fasted five days, no water; Dec. 1 blood was obtained from right femoral artery, blood and serum kept on ice for 4 days, then inoculated and plated.

37° C.	Immediate.	1 hr.	3 hrs.	6 hrs.	28 hrs.	3 days.
Blood	3831	572	84	14	∞	∞
Serum	2046	471	104	21	∞	∞
21° C.						
Blood	2814	1653	922	336	?	
Serum	1552	1701	662	381	651	

Exp. D[2]. The same dog well fed since last operation, Dec. 17, blood from left femoral artery, blood and serum 4 days in ice-chest, then inoculated and plated.

37° C.	Immediate.	1 hr.	3 hrs.	6 hrs.	28 hrs.
Blood	1807	286	81	23	∞
Serum	954	265	54	3	∞
21° C.					
Blood	1219	948	579	286	328
Serum	3768	588	445	312	1817

In this experiment the dog fasted first and was then fed well for over two weeks before blood was again taken; the result obtained was the same as in the foregoing experiments, and, to say briefly, as in all the previous experiments. Our hypothesis did not stand the test of the experiments; these have shown conclusively that five days' fasting did not affect the bactericidal power of the blood as tested with the typhoid bacillus, in the slightest degree. There seemed to be no difference in the bactericidal action of the blood, no matter whether it was taken from a well-fed or even over-fed dog or from an animal in a state of complete inanition.

We might perhaps explain this surprising fact in the following manner: According to Buchner the degree of the bactericidal action of the body-fluids depends upon two opposing factors; the alexines, which are generated by the leucocytes, and the nutritive character of the fluid. There can be no question that fasting impoverishes the blood. If we now assume that feeding or fasting increases or diminishes the nutritive capacity of the blood to the same degree as hyper- or hypoleucocytosis is obtained, it would then be quite natural that the bactericidal action should always remain the same, for when numerator and denominator are multiplied or divided by the same number, the value remains unchanged.

As to any apparent incompatibility of our results with the common assumption that inanition favors infection, we must in the first place

bear in mind that our present results apply only to the bactericidal action of the blood of the dog upon the typhoid bacillus; with other animals and with other microörganisms the conditions may be different. Canalis and Morpurgo could not by starvation influence the immunity of the rat from anthrax, although the immunity of the pigeon was abolished. On the other hand the bactericidal properties of the blood are not the only defensive elements of the body against infection. Other factors, notably the body cells, are concerned, and it may well be that the powers of resistance of the latter may suffer to a considerable measure through the starvation of the animal.

GLYCOSURIA IN DIPHTHERIA.

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AND

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The occurrence of a transitory glycosuria in diphtheria has been noted by only a few observers. Sannét† in his monograph on diphtheria says: "The analyses made by Fouris to determine the presence of sugar (in the urine) have given only negative results." Dickinson‡ reports: "I have examined many cases of croup in which the breathing has reached the extreme of difficulty without finding a trace of sugar in the urine, with one exception—a girl seven years old." Simon§ in his Clinical Diagnosis speaks of transitory glycosuria in diphtheria. In a personal letter Dr. Simon says: "A few years ago while examining a series of urines with the phenylhydrazin test I accidentally came across a urine from a case of diphtheria in which the osazon needles were present in fairly large numbers. Two years ago I had occasion to examine the urine of thirty-two cases of diphtheria, all of which were of moderate severity. A positive reaction was obtained with the phenylhydrazin in four. Three of these four were treated with antitoxin. The amount of sugar in each case was small and persisted, if I remember, only one to two days." A Rumbold|| remarks that in diphtheria during convalescence glycosuria has been demonstrated on rare occasions, but gives

* Dr. Hibbard died as the result of an elevator accident in St. Louis in July, 1898.

He was graduated from the Harvard Medical School in 1895 and subsequently served for two years as Assistant Resident Physician in the South Department of the Boston City Hospital. He published several valuable contributions to our knowledge of the infectious diseases of children. He was an enthusiastic and untiring student, who gave promise of unusual success as a clinical investigator.—W. T. C.

† Gillis' English translation, 1886, p. 185.

‡ W. H. Dickinson. Diseases of the kidney and urinary derangements, Pt. I; Diabetes, p. 20, London, 1875.

§ Charles E. Simon. A manual of clinical diagnosis, p. 369. Philadelphia and New York, 1896.

|| *Wien. klin. Wochenschr.*, 1894, p. 83.

no reference and no observations. Coleman* speaks of a transitory glycosuria in diphtheria.

Paul Binet† found in diphtheria a positive reaction with the phenylhydrazin test in 29 out of 70 cases. Dividing these into mild and severe forms, there were 32 of the former with only 2 positive reactions, while of the 38 severe cases there were 27 with positive result. Of 19 cases of laryngeal diphtheria 11 showed the characteristic osazon crystals. With Fehling's test 37 of the 70 cases gave a green or a greenish-yellow reaction. The glycosuria was frequently associated with albuminuria.

The following observations are the result of about one thousand analyses of the urine for sugar by Fehling's test from diphtheria patients of the South Department of the Boston City Hospital from October 1, 1897, to January 1, 1898. Specimens of urine were examined from all old patients in the wards and from new ones as they were admitted. The analyses were made every three to five days. A daily test was usually made in those cases that showed a positive reaction or were very sick.

TESTS.—*Fehling's:* 7 cc. of the mixed solution were boiled in a test tube and to this 1 cc. of cold urine were added. If the urine contained albumin, this was first separated by heat and filtration. The urine and Fehling's solution were well shaken and allowed to stand until the next day without further heating. The presence of a yellowish precipitate in the bottom of the tube was considered as a positive reaction. In any instance where there was any question as to the result of the reaction it was called negative.

Most of the positive results with Fehling's method were confirmed by the *phenylhydrazin test*, which was performed in these observations in the following manner:‡ To 7 cc. of filtered urine, free from albumin, 3 or 4 drops of phenylhydrazin and the same quantity of 50 per cent acetic acid were added. The mixture, after being well shaken in a test tube and kept in a water bath for at least one hour, was allowed to settle until the next day. The brownish sediment was examined under

* Article on Diabetes in Loomis and Thompson's System of Practical Medicine by American Authors. Vol. iii, p. 829. New York and Philadelphia, 1898.

† *Rev. méd. de la Suisse Rom.*, 1892, xii, 69.

‡ Foster's Physiology, part v, p. 104.

the microscope and the presence of the characteristic phenylglucosazon crystals made a positive reaction.*

In the following analyses only those cases that gave a positive result both by Fehling's and by the phenylhydrazin tests were considered as instances of glycosuria. Doubtless many of the urines, not in the table, which reduced the copper solution contained glucose, but for various reasons the phenylhydrazin test was not applied to some of them.

Table I gives the results of Fehling's tests in 230 cases of diphtheria, in all of which the Klebs-Loeffler bacillus was found. Some of these patients were well along in convalescence; other urines were examined only once or twice. The cases are classified according to the presence or absence of pseudo-membrane and its location.

TABLE I.—Results of Fehling's Test.

	RECOVERED.			DIED.			TOTAL.		
	No. of cases.	No. of positive reactions.	Percentage of positive reactions.	No. of cases.	No. of positive reactions.	Percentage of positive reactions.	No. of cases.	No. of positive reactions.	Percentage of positive reactions.
No membrane	22	0	0	1	0	0	23	0	0
Membrane on tonsils	98	8	8	3	1	33	101	9	9
In nose	5	2	40	0	0	..	5	2	40
In larynx	11	2	19	2	2	100	13	4	31
Tonsils and nose	45	12	27	6	5	83	51	17	33
Tonsils and larynx	19	10	53	5	4	80	24	14	58
Nose and larynx	5	3	60	1	1	100	6	4	67
Tonsils, nose and larynx	3	2	66	4	4	100	7	6	86
Total	208	39	19	22	17	77	230	56	25

It is seen that in the cases without false membrane there was no positive reaction, and that, in general, the more extensive the membrane, the more frequently a positive reaction was found. A reaction was noted in 19 per cent of the recoveries, in 77 per cent of the fatal cases and in 25 per cent of all the cases.

In a second series of 96 cases the positive reactions by Fehling's

* We wish to thank Dr. J. Bergen Ogden, Assistant in Clinical Pathology in the hospital, for his help and advice in these observations.

method were confirmed by the phenylhydrazin test. These analyses were begun during the first week of the patient's stay in the hospital, and were followed for at least a fortnight by as many as three examinations. In other words, those cases of Table I are omitted in which a reaction by Fehling's test was not confirmed by phenylhydrazin, and also those not examined within the first week of the illness.

TABLE II.—RESULTS OF FEHLING'S AND THE PHENYLHYDRAZIN TESTS.

	RECOVERED.			DIED.			TOTAL.		
	No. of cases.	Cases with sugar.	Percentage of sugar cases.	No. of cases.	Cases with sugar.	Percentage of sugar cases.	No. of cases.	Cases with sugar.	Percentage of sugar cases.
No membrane.........	10	0	0	0	0	......	10	0	0
Membrane on tonsils..	37	6	16	2	1	50	39	7	18
In nose..............	2	1	50	0	0	......	2	1	50
In larynx	4	2	50	1	1	100	5	3	60
Tonsils and nose......	21	8	38	2	2	100	23	10	44
Tonsils and larynx....	9	5	55	1	1	100	10	6	60
Nose and larnyx......	3	3	100	1	1	100	4	4	100
Tonsils, nose and larynx	1	0	0	2	2	100	3	2	66
Total...........	87	25	28	9	8	88	96	33	34

Table II shows, in an even more marked degree, the same results as those in Table I, and also that a transitory glycosuria, of at least as much as 0.5 gramme of glucose to the litre of urine,* is not at all uncommon in the course of diphtheria. There were 15 cases which gave a positive Fehling's reaction that are omitted from Table II, as a phenylhydrazin test for some reason was not made; so that the percentage given is doubtless too low. The greater the surface involved by the diphtheritic process the greater the frequency of sugar. Again, a glycosuria is three times as common in the fatal cases as in those that recover.

The time at which Fehling's test was first positive varied from the second to the eighteenth day. In 35 cases a positive reaction was found on the first analysis, and in 21 on the second, or on some subse-

* F. D. Beane. The comparative clinical value of several tests for glucose in the urine. *New York Med. Jour.*, 1893, lvii, 12.

quent examination. In two cases five negative results were obtained before a positive one. In the 44 positive cases that were tested by Fehling's solution within the first week of the illness, the reaction was found within that time in all but 8, and in the latter it was obtained during the second week. In one instance after a reaction had appeared on the eighth day it was followed by six negative results, and then on the twenty-first day by another positive reaction, confirmed by the phenylhydrazin test. In another case after a glycosuria had been shown with Fehling's solution, and again on the seventh and fourteenth days three negative results followed: a positive result appeared on the twenty-second and twenty-sixth days, and a negative on the twenty-eighth day.

The duration of the glycosuria varied from one day to several weeks. Seven patients left the hospital with sugar still present in the urine. In a case of unresolved pneumonia, following a triple attack of laryngeal diphtheria that had been in the hospital for five months, glycosuria was found on ten successive analyses during the sixth month of the illness.

An immediate reaction with Fehling's test occurred 16 times in the 56 positive cases. In the others the reaction was seen at the end of 24 hours. In three cases the immediate reaction was observed from one to three days after one that appeared only at the end of a day. There were three quantitative estimations with Fehling's solution, with the following results: 1.5, 2, and 3.3 per cent, the first two of these being in fatal cases. Of the 16 patients with an immediate reaction eight or 50 per cent died. It would thus seem that an immediate reaction with Fehling's test is of serious prognostic import, more so than the presence of albumin in the urine of a diphtheria patient.

The relation of glycosuria to albuminuria is shown in Table III, which gives the number of cases in which the reaction for sugar was absent, the number in which it was obtained and the percentages.

TABLE III.—Relation of Glycosuria to Albuminuria.

Albumin.	No sugar.	Sugar.	Percentage.
None	58	0	0
Slight trace	139	16	10
Distinct trace	25	11	31
0.125 per cent or more	2	8	80

These results confirm the observation of Binet that the glycosuria of diphtheria is usually associated with albuminuria, without there being necessarily any definite relation between them, and also that sugar is oftener present in the severer cases of albuminuria.

Some additional observations on scarlet fever both with and without diphtheria were made by Mr. C. H. Dean, the clinical clerk in the scarlet fever wards, who made 920 Fehling's tests in 129 cases of scarlet fever. Of 93 patients, in whom no diphtheria bacilli were found, 16 gave positive reaction once. One of these patients was pregnant, and therefore this case should be eliminated, as the reduction was probably due to lactose, the phenylhydrazin test giving phenyllactosazon crystals. This makes the positive reactions about 16 per cent. 4 patients had such suspicious looking membranes that they received antitoxin, and the urine of 3 of these gave a yellow reaction. Of 36 cases of scarlet fever with positive cultures of diphtheria bacilli from the nose or throat, 11 or about 30 per cent gave a positive reaction. In the last series of 37 cases, 17 received antitoxin and 6 of these showed a reduction of the copper, which gives about the same percentage of reactions as in the cases which did not receive antitoxin. All the positive results in scarlet fever occurred only once, save in 3 cases which also had diphtheria. In one the reaction appeared on two occasions with an interval between them of 22 days. In the other case it was found on three successive examinations during a period of 10 days. In the third, a fatal case, it was present in the last two days of life. It is to be regretted that these reactions were not confirmed by the phenylhydrazin test.

In the scarlet fever patients who also had diphtheria bacilli, the percentage of positive reactions was about twice that found in scarlet fever alone. This observation would seem to indicate that the existence of diptheria is a causal factor in the production of a reducing agent in the urine.

Eliminating the case of lactosuria, there were 21 scarlet fever patients who received antitoxin, and 107 who did not, with 9 positive reactions or 43 per cent in the former, and 17 reactions or 16 per cent in the latter. In other words, a reaction was over two and a

half times more frequent in those patients who received antitoxin than in those who did not. From this it would appear that the administration of antitoxin contributed to the formation of a substance in the urine which reduces the copper in Fehling's solution.

9 cases of measles were examined several times by Fehling's test, each with negative results, save in one instance, a patient who was nursing a child. The reduction here was doubtless due to lactose, as no reaction was obtained with the phenylhydrazin test.

In sixty-eight cases of tonsillitis the urine was examined on one or more occasions. A positive result was obtained only once, but unfortunately in this instance, no confirmatory phenylhydrazin test was made.

As almost every diphtheria patient received more or less antitoxin the question arose, Was this method of treatment responsible for the transitory glycosuria found in our patients? In order to clear up this point the urine of 20 patients who had false membrane and the Klebs-Loeffler bacillus was examined prior to the serum injection with the following results:

Fehling's test.—15 cases negative: 1 urine gave a slight reduction in 24 hours: 3 cases gave a good reduction in 24 hours.

Phenylhydrazin test.—10 cases had no crystals: 6 cases had a few osazon crystals: 4 cases had many crystals.

The percentage of positive Fehling's reactions in these few cases is about the same as in Series I, while the positive results with the more delicate tests, as was to be expected, are more frequent than in Series II. These results warrant the conclusion that the antitoxin treatment cannot be considered as the full explanation of this symptom in diphtheria.

To decide whether diphtheria antitoxin injected into patients may produce a glycosuria 10 individuals were given antitoxin in whom the two tests for glucose had proved negative before the injection, and the urine was examined afterward. The results of these observations are given below in detail:

No. I. Male 19 years old. Admitted Dec. 30 with tonsillitis. At 4 and 10 P. M. Fehling's and phenylhydrazin tests were negative. At

11 P. M. given 20 cc. antitoxin. Examinations at 4 and 9 A. M. the following morning were negative by Fehling's, but there were a few doubtful sheaf-like crystals by the latter test. Dec. 31, at 9.30 A. M., given 20 cc. antitoxin. At 4 P. M. and 1 A. M., Jan. 1, Fehling's test was negative. At 9 A. M. a very few doubtful osazon crystals. Same found on Jan. 2. The results of this experiment are called negative as the crystals were so few and of a questionable nature.

No. II. Male 22 years old. Admitted Nov. 5 with a severe diphtheria of throat; given first day 20 cc. antitoxin; the urine next morning was positive to Fehling's test. From the second to the fourth day received 55 cc. more antitoxin. On the fifth day both Fehling's and the phenylhydrazin tests were positive. On the eighth day both were negative. During the following month had eight negative results by Fehling's test. On Dec. 16 was given 20 cc. antitoxin; the urine was examined every 12 hours for the next 5 days, then once a day for 5 days by both tests, with negative result.

No. III. Boy, 13 years, admitted Nov. 18 with tonsillar diphtheria. He received 60 cc. antitoxin during the first two days. Six examinations with Fehling's solution were made in the first 4 weeks with negative result. Dec. 20 phenylhydrazin test negative. Given 20 cc. antitoxin on this date. The examination by Fehling and the phenylhydrazin tests were negative on the two days following.

No. IV. Girl, 10 years, admitted Nov. 15 with diphtheritic tonsillitis, so mild that antitoxin was not given. Four examinations in 3 weeks, by Fehling's test, were negative. On Dec. 28 given 20 cc. antitoxin. The phenylhydrazin test just before antitoxin was given was negative, but six hours afterwards positive; there were numerous typical crystals. The next three days many crystals of osazon appeared, and after that for a week only a very few or none were seen. Fehling's tests were negative, with possibly one doubtful reaction on Jan. 5.

No. V. Girl, 7 years, admitted Oct. 30 with a mild attack of diphtheria; was given no antitoxin. On Nov. 3 Fehling's and the phenylhydrazin tests were both positive. From Nov. 6 to Dec. 28 there were 8 negative copper tests and on the last date there were no osazon crystals. On Dec. 28 were given 20 cc. antitoxin. The following morning there was a slight greenish-yellow discoloration of the Fehling's solution and numerous phenylglucosazon crystals were seen. The following 12 examinations by Fehling's test were all negative save one doubtful one. The phenylhydrazin test was positive for the next 6 analyses, then negative.

No. VI. Girl, 7 years. Admitted Dec. 7 with slight nasal diphtheria; received no antitoxin. On the next day had an immediate reaction to Fehling's test, followed by five negative results during the next week. Dec. 18 given 10 cc. antitoxin. At that time and the next day the urine was negative to both the Fehling's and phenylhydrazin tests, but on the following day it was positive in the morning and evening to the phenylhydrazin test and afterwards negative. Fehling's tests were all negative.

No. VII. Girl, 7 years, admitted with a mild attack of scarlet fever on Dec. 27. Urine examined daily from Jan. 13 to 19. Negative by both tests. On the last date given 20 cc. antitoxin. Analyses twice a day for two days, then daily for five days with negative results.

No. VIII. Boy, 6 years, taken ill with scarlet fever Dec. 15. Similar tests were made, on the same date, as in No. VII. The morning after the antitoxin injection Fehling's test was positive and there were many phenylglucosazon crystals seen. There was a slight reaction that evening, afterwards negative.

No. IX. Girl, 9 years old, admitted with scarlet fever, Dec. 29. Experiment same as in No. VII. Results negative.

No. X. Boy, 7 years old, admitted Dec. 18 with scarlet fever. In this case, as in No. VII, Fehling's tests were positive, but with phenylhydrazin the results were negative.

A summary of the results of these experiments by the two tests are:

Case No.	Fehling's.	Phenylhydrazin.
I.	Negative.	Negative.
II.	Negative.	Negative.
III.	Negative.	Negative.
IV.	Negative.	Positive.
V.	Doubtful.	Positive.
VI.	Negative.	Positive.
VII.	Negative.	Negative.
VIII.	Positive.	Positive.
IX.	Negative.	Negative.
X.	Positive.	Negative.

Fehling's test was twice positive and the phenylhydrazin four times. Thus it would appear that antitoxin injections produce at times a glycosuria, but that usually it is so slight that it is shown only by the most delicate tests; on the other hand sugar is occasionally present in a sufficient quantity (0.05 per cent at least) to reduce Fehling's solution. This agrees with what was noted in regard to the

scarlet fever patients who received antitoxin (pp. 142-3). It should be added that this glycosuria lasts only for a few days and should not be considered as an argument against the use of antitoxin. The significance of a transitory glycosuria after antitoxin would appear to be about the same as that belonging to the alimentary glycosuria which may occur after the ingestion of certain foods or drugs, or after drinking beer, as Krehl* has recently shown.

Binet† gives as the cause of the glycosuria in diphtheria an "asphyxie toxique," due for the most part to a simple mechanical obstruction of the respiratory passages. Von Bager‡ reports an instance of glycosuria in a patient troubled with a nasal obstruction which disappeared after an operation. Tyson§ says: "Embarrassed respiration, whether due to strangulation or inhalation of irrespirable gases is capable of producing glycosuria in dogs and rabbits, although the symptom has rarely been shown in those conditions in human subjects." Of 19 cases of intubation, that is, of patients with severe laryngeal obstruction, fourteen, or 73 per cent, had glycosuria. This result would make somewhat plausible the supposition that diminished oxidation in consequence of the greater or less interference with respiration has a causal connection with the glycosuria of diphtheria. It seems to us, however, reasonable to attribute this symptom in diphtheria to the action of the toxins of the Klebs-Loeffler bacillus. Interference with respiration may be an occasional cause, but it will not account for those cases in which there is no trouble with the breathing. Inasmuch as irritation of the vagus nerve and various lesions of the nervous system may be followed by glycosuria, and as degenerative changes in the peripheral and central nervous system are found in most cases of diphtheria, it is possible that these changes in the nervous system in diphtheria may cause glycosuria. Or it may be that the diphtheria toxins may act as phloridzin‖ does in producing glucose in the urine.

* *Centralbl. f. inn. Med.*, 1897, No. 40.

† Loc. cit., p. 87.

‡ *Centralbl. f. d. Krankheiten d. Harn- u. Sexual-Organe*, vi, 61.

§ A treatise on Bright's disease and diabetes, p. 239, Philadelphia, 1881.

‖ Lectures on certain aspects of diabetes. by T. B. Futcher in the *New York Med. Jour.*, Nov. 6, 1897.

CONCLUSIONS.

(1) There is a transitory glycosuria in diphtheria, which is found frequently in the severe cases and is usually present in the fatal ones.

(2) This glycosuria is often associated with albuminuria.

(3) Injections of diphtheria antitoxin are occasionally followed for a few days by a slight glycosuria.

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HÆMORRHAGIC SEPTICÆMIA IN MAN DUE TO CAPSULATED BACILLI.*

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In this paper it is my purpose to consider only those cases of septicæmia accompanied by hæmorrhages into the skin, the serous membranes, and usually into the liver, spleen, kidney, heart, stomach and intestines in which capsulated bacilli have been found as the only, or, at least, the most numerous and important micro-organisms present.

These restrictions remove from our consideration cases of primary septicæmia with hæmorrhages, as well as the numerous instances of secondary hæmorrhagic septicæmia occurring in variola, typhoid fever, scarlatina, rheumatism, measles, tuberculosis, endocarditis, nephritis, puerperal fever, omphalitis, and the like, due for the most part to streptococci, staphylococci, or various bacilli, examples of which have been reported by Klebs (16), Hlava (14), Babès (2), Martin de Gimard (19), Claisse (9), Legendre and Claisse (18), Tavel and de Quervain (21), Kamen (15), Neumann (20) and others. The so-called purpura anthracosa and purpura scorbutica are also, of course, excluded.

It is difficult to say by whom the class of cases with which we have to do was first described. Although some of the early examples reported

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by Ceci (7) in 1881, and by Klebs (16) in 1887, and that of Watson Cheyne (8) in 1883, in which bacilli were found, probably come within the scope of this article, the descriptions of the microscopical lesions and of the bacteria found are too scanty to warrant their consideration here.

In 1887 Bordoni-Uffreduzzi (6) described three cases of a peculiar septicæmia, fatal in from two to four days, with hæmorrhage into and congestion of the internal organs. In each instance he found the same micro-organism, a pleomorphic capsulated bacillus pathogenic for dogs, mice, rabbits and guinea-pigs. He called it *Proteus hominis capsulatus.* It differs in many ways from the Proteus group of Hauser. This organism was about the size of the anthrax bacillus, capsulated, and stained by Gram. It was found in numbers in the larger blood-vessels and among the tissue elements, but not in the capillaries.

It is thought best to exclude from our list the case described by Foà and Bonome (11), who report finding a bacillus identical with Proteus vulgaris (Hauser) in the blood and in the organs of a man dead of ileus with extensive hæmorrhagic infiltration of the small intestine and the corresponding mesentery and with thrombosis of the mesenteric vein. It is not at all clear that the "hæmorrhagic infiltration" was other than hæmorrhagic infarction of the intestine.

Banti (5) in a case of acute hæmorrhagic infection found in pure culture a bacillus which he calls *Proteus capsulatus septicus,* and which he thinks may be identical with Proteus hominis capsulatus of Bordoni-Uffreduzzi. Banti's bacillus appears to be more like the bacillus of Friedländer than was the organism of Bordoni-Uffreduzzi. The latter is regarded by Foà as Proteus vulgaris (Hauser) and not as a new bacillus.

Babès (1) in 1889 described three instances of death in children from hæmorrhagic septicæmia, with stomatitis, angina, bronchitis, anæmia and fever, in the organs of whom he found a short, non-motile, capsulated bacillus, which stained poorly with the aniline dyes and by Gram, did not form spores, was facultatively anaërobic, and grew feebly on gelatine, which was not liquefied. On agar there were small transparent drops which later turned to a whitish yellow color, and were without sharply defined margins. On potato, whitish drop-like colonies formed. Bouillon was made cloudy. Mice inoculated with this bacillus died with hæmorrhagic septicæmia; rabbits, killed by it, showed hæmorrhage into all the organs. Sterilized cultures caused multiple hæmorrhages when injected into animals. For guinea-pigs and dogs it was but feebly pathogenic.

Tizzoni and Giovannini (22) in 1889 reported three cases of hæmor-

rhagic infection in which they found a capsulated bacillus. They were in children with impetigo contagiosa and two of them were rapidly fatal. One child came to autopsy and showed œdema and hæmorrhages into the skin, hæmorrhages in the serous membranes, lymphatic glands and various internal organs. In the kidneys and liver there were areas of marked tissue degeneration and leucocytic infiltration. The bacillus found was pleomorphic, capsulated, often with rounded ends, stained by Gram and was pathogenic for dogs, rabbits and guinea-pigs, but not for white mice or for pigeons, even in large doses. In the cutaneous lesions and in some of the organs Staphylococcus pyogenes aureus was also present.

In 1891 Kolb (17) reported five cases of idiopathic purpura hæmorrhagica, three of which died in from two to four days. Cultures made from blood obtained from a vein during life remained sterile. Cultures from the various organs of the fatal cases gave always the same bacillus in pure culture. This bacillus was demonstrated in sections stained by the Kühne and the Gram-Weigert methods, being especially abundant in the spleen; it was sometimes found in the blood and lymph vessels, sometimes in the interstitial tissue of the hæmorrhagic areas and in the lymph spaces. It had usually rounded ends and was surrounded by a thin capsule, especially in the animal body. It was partly decolorized with Gram's method. The bacillus was very pathogenic for field mice, for white mice and for rabbits; less so for dogs. Guinea-pigs were affected only by large doses, while pigeons showed only local necrosis at the seat of inoculation. Hæmorrhages were found in the animals after inoculation with dead and filtered, as well as with living, cultures.

In 1891 Babès and Oprescu (4) described a fatal case of hæmorrhagic septicæmia simulating typhus fever. From the organs and urine they obtained a capsulated bacillus in pure culture. The patient, a male, aged 22, had had marked hæmorrhages into the skin. At the autopsy the tissues of the neck and of the mediastinum were œdematous, and there were hæmorrhages into the skin and serous membranes. Tissue degenerations were found in the liver, spleen and kidneys.

The bacilli, which were found in all the organs, varied much in size; they were often in pairs, were surrounded by capsules and stained poorly by Gram. The organism was pathogenic for rabbits, guinea-pigs, mice and pigeons, the animals dying with hæmorrhagic septicæmia.

In 1893 Babès (4A) reported several cases of hæmorrhagic infection, associated especially with hæmorrhagic bronchitis, and referred by him to various micro-organisms, both cocci and bacilli.

Von Dungern (10) in 1893 reported a fatal case of hæmorrhagic sep-

ticæmia in a child, two weeks old, in whose organs was found a capsulated bacillus. There was no history of syphilis or hæmophilia. Thirteen days after birth the child was taken ill with hæmorrhage from the right ear, nose and mouth. Later there appeared petechiæ over the whole body. The autopsy showed hæmorrhages in various organs, in the subcutaneous tissue of the thighs, in the abdominal cavity, and in the umbilicus. Cultures from the thrombosed umbilical artery showed a short, plump bacillus, 1 to 2 μ long and half as broad, surrounded by a capsule. This bacillus was very pathogenic for white mice, guinea-pigs and rabbits, causing a fatal hæmorrhagic septicæmia with a fibrino-purulent exudation into the serous cavities.

To the foregoing cases I wish to add two others which came under my observation at St. Joseph's Hospital, Baltimore.

In June, 1892, five persons who had been working as berry-pickers, near Annapolis, Maryland, were taken ill and came to Baltimore for treatment. One died several days later under the care of a physician who gave a death certificate for "malaria." Two others came to St. Joseph's Hospital; the remaining two could not be traced. The two patients admitted to my service at the hospital were suspected of having typhus fever. One died a few hours after admission; the other died at the Quarantine Station some ten days later.

Case I. John Y., married, aged 51, a Pole, was admitted to St. Joseph's Hospital at 3 p. m., June 13, 1892, complaining of fever and weakness.

Family history.—Father and mother dead, cause unknown. One brother and sister living, none dead. One son and three daughters living and well. Knows nothing of his grandparents. His wife was taken with the same symptoms the day before he was affected.

Personal history.—Healthy as a child. Eleven years ago had typhoid fever. No other illness.

Present illness.—Seven days ago, while picking strawberries in the hot sun (the weather was excessively hot at that time) his head became very hot and painful, and shortly afterward he was seized with pain in the epigastrium. These symptoms came on suddenly, the patient having felt perfectly well before their appearance. No history of a chill was obtained, but the patient felt hot and feverish from this time on. On the next day, June 8, his nose bled four times. No further history could be got from the patient, except that his wife and three of his fellow-workers were similarly affected.

On entrance into the hospital, the axillary temperature was 102.4° F.; pulse 90, regular, of fair volume, and not dicrotic; respirations 30. He was seen by me two hours after his entrance, when the following note was made: Large, well-built, muscular man; skin dusky, warm and dry; face heavy, apathetic; he answers questions slowly but intelligently. Pupils of normal size and react to light; conjunctivæ slightly suffused. Tongue covered on the dorsum with thin white fur, the margins bright red, the papillæ swollen. No œdema. Surface of the skin covered with a purplish macular eruption, which on the anterior surface of the chest resembles very light-colored freckles. On the back they are very numerous and red in color. On both arms, the buttocks, thighs and legs, always best marked on the extensor surfaces, is a diffusely spread, dark red, slightly papular eruption, which fades on pressure and slowly returns on its removal. It is somewhat confluent in places, but is never crescentically arranged. The individual papillæ have no definite shape. On the legs, over the tibiæ and ankles, are numerous purplish petechiæ, resembling spots of purpura hæmorrhagica. Examination of lungs negative. Cardiac dulness slightly increased; heart sounds normal. Examination of liver negative. Splenic dulness increased; spleen not palpable. No rose spots on abdomen. No gurgling or tenderness in right iliac fossa.

June 14, 10 a. m. Temperature 104.5° F. Pulse continues strong. Patient restless and at times delirious during the day. Temperature reached its highest point, 105.4° F., at 8 p. m. Urine dark red, acid, sp. gr. 1024. No albumin, sugar or casts. Moderate increase of polynuclear leucocytes in blood. No malarial parasites found. At 9 p. m. patient was taken to the Quarantine Station, and passed from my observation.

The quarantine officials informed me that the fever and cutaneous hæmorrhages persisted, and that death occurred ten days after his removal from the hospital. The report of the coroner's physician, Dr. Keirle, who made the autopsy, states that there were hæmorrhages into the skin and serous membranes, with marked congestion of the lungs, liver, spleen, heart, kidneys, brain and cord, and acute parenchymatous degeneration of the heart, liver and kidneys. No cultures were made.

Case II. Julia Y., a Russian Pole, 45 years old, was admitted to St. Joseph's Hospital with her husband (Case I) June 13, 1892, complaining of fever and epigastric pain. Family history negative; personal history good.

On June 7, while picking strawberries in the hot sun, she was suddenly seized with great pain in the head, epigastrium and chest. She

felt very hot all over, but especially in the head. The same day she broke out with a slightly papular eruption which was spread diffusely over the body. No history of a chill was obtained. She was seen by me two hours after her entrance, when the following note was made: Well-built woman, of medium height. Conjunctivæ of both eyes, especially of the lower lids, injected. Both corneæ slightly clouded, pupils moderately dilated. Skin of the nose and face somewhat swollen, but not œdematous. Tongue swollen and painful to the touch, its dorsum covered with yellowish white fur, the papillæ prominent, edges red. Small whitish ulcers on the inner side of each lip. Patient lies on the back, is dull and apathetic, answers questions with difficulty. Temperature on admission 101.4° F.; pulse 126, slightly irregular, weak, low tension, not dicrotic. Respirations 45, shallow, costo-abdominal. No enlargement of lymphatic glands. Skin warm, moderately dry; its surface covered everywhere with thick and diffusely spread papular eruptions. In some places, as on the anterior surface of the chest, the papules are small, dark red, irregularly shaped, about the size of a pin's head, not fading on pressure. In some places there are true petechiæ; in others, particularly on abdomen, surface covered with macular eruption, appearing as a fine dark mottling distributed in irregular streaks. Papular eruption predominates over back, anterior surface of thighs and buttocks. On arms and forearms, best marked over deltoid regions and posterior surface of elbow joint, is a diffuse, red, thickly scattered, small papular eruption, very irregular in shape, and in places somewhat concentrically arranged, very slightly modified by pressure Skin of thighs, legs, and anterior surfaces of ankles and feet presents a widespread petechial eruption. On chest numerous small sudaminal vesicles. Skin over lower extremities covered with sweat. Examination of heart and lungs negative. Liver dulness begins at lower border of 6th rib in mammary line, and extends 3 cm. below costal margin. Edge of liver palpable, round and smooth. Splenic dulness noticeably increased; edge of spleen not easily palpable below costal margin. Much tenderness over abdomen, which is everywhere resonant; no special tenderness or gurgling in right iliac fossa.

June 14, 10 a. m. Patient decidedly worse this morning; pulse 108, weak, of poor volume, irregular and dicrotic; temperature 100.4° F., respirations 30. Patient was restless and delirious throughout night, and was kept in bed with difficulty. Bowels constipated. Urine scanty; specimen drawn with catheter is pale yellow, acid, specific gravity 1023, free from albumin, sugar, and casts. No characteristic diazo-reaction; no hæmaturia or hæmoglobinuria. Death occurred at 4.15 p. m., pre-

ceded by great rise of temperature (109.6° F. at 1 p. m.). rapid, weak, dicrotic pulse and persistence of the eruption.

AUTOPSY (5 hours after death). Anatomical diagnosis: Haemorrhagic septicæmia; acute splenic tumor; fluid blood in the heart and all the blood-vessels. Acute congestion of the lungs, liver, spleen, kidneys, pancreas, stomach and intestine. Cloudy swelling of liver and kidneys. Fatty degeneration of heart, liver and kidneys.

Rigor mortis present. On skin of abdomen there is a fine macular eruption of a mulberry hue. Here and there are scattered petechiæ and several "taches bleuâtres." On posterior and inner surfaces of arms and lower extremities and on back purplish-blue post-mortem discoloration. On anterior surface of upper and lower extremities numerous large and small petechiæ, which do not disappear on pressure. Superficial lymphatic glands not swollen. Muscles dark red.

Brain.—Dark fluid blood, without clots, in sinuses. Hyperæmia and œdema of brain and meninges. *Abdomen:* Moderate hyperæmia of peritoneum. A few scattered subperitoneal ecchymoses. Intestinal surface dark red, mesenteric glands swollen and dark red. *Thorax:* Marked hyperæmia and numerous small subpleural ecchymoses, especially over lower lobes. Lungs very hyperæmic and œdematous. Muco-pus in bronchi. Clear serum in pericardial cavity. Small ecchymoses beneath epicardium. Dark fluid blood in all cardiac cavities. Myocardium flabby and pale. Valves normal. Arteries normal, contain dark, fluid blood. *Liver:* Much swollen, very hyperæmic, of mottled dark red and opaque grayish color, and soft consistence, without visible focal necroses. It presents small scattered hæmorrhages. Gall bladder contains thick, dark greenish bile. *Spleen:* Swollen, very soft, almost diffluent, and dark red. Trabeculæ and Malpighian bodies invisible. *Kidneys:* Very much swollen, especially the cortex, of a dark red color, and softened in consistence. Cortical striæ obscure, opaque: Malpighian tufts dark red and prominent; pyramids very hyperæmic. Capsule not adherent. *Pancreas* and *adrenals:* Show no especial change, except congestion. *Stomach* and *intestine:* Deeply injected along their mesenteric border. Gastric and intestinal mucosæ bright red, deeply congested. Peyer's patches in upper portion of ileum swollen and, with the solitary follicles, red. Peyer's patches just above the ileo-cæcal valve somewhat swollen and present a shaven-beard appearance. No ulceration. *Uterus* of normal size, with soft, red and congested mucosa. Ovaries and Fallopian tubes normal. *Bladder:* Contains blood-stained urine and its mucosa is red and injected. *Thyroid:* Twice the normal

size, being symmetrically swollen, is dark red and very much congested. Its consistence is soft.

MICROSCOPICAL EXAMINATION.—Fresh frozen sections and sections of tissues hardened in Flemming's solution, in alcohol, and in Müller's fluid were studied. Sections of hardened tissues were stained by safranin, hæmatoxylin and eosin, lithium carmin, Weigert's fibrin stain, and methylene-blue and eosin.

Liver.—There was widespread fatty degeneration of the hepatic cells, as well as of the endothelial cells of the intralobular capillaries and of some of the large veins. The liver cells were swollen and granular; many contained much yellow pigment. The capillaries were everywhere dilated, being filled with red corpuscles, among which were many large mononuclear and polymorphonuclear leucocytes. The central veins of the lobules and the neighboring capillaries were widely dilated, the liver cells about them being flattened by pressure. The capillary endothelium was swollen. All the branches of the portal and hepatic veins were dilated. In some of the larger veins were seen many long cells with elongated nuclei. The hepatic veins contained liver cells, singly and in clumps of two, three or even more. There were a few small intralobular areas of infiltration with lymphoid cells, some short fusiform cells, and some polymorphonuclear leucocytes. There was some increase of the interlobular connective tissue. Larger and smaller areas of hæmorrhage were present within the lobules. In some places only a few red corpuscles were seen among the liver cells, while in others the hæmorrhages occupied as much as the third of a lobule. Both in the veins and in the capillaries were to be seen short and long bacilli, occurring both singly and in small groups. Here and there they occurred also free in the tissue, being numerous in the areas of hæmorrhage and of round-cell infiltration. No capsules were definitely detected, and the bacilli were never observed within cells.

The *heart* showed fatty degeneration of the muscular fibres, with great dilatation of the capillaries and small veins, in both of which bacilli could be seen.

Sections made from several portions of each *lung* showed engorgement of all the blood-vessels with blood. In the lower lobes there was marked œdema, with great dilatation of the capillaries and veins. There was no inflammatory exudation in the air vesicles. In some of the small arteries and dilated capillaries liver cells were seen. Bacilli were found in the blood-vessels but not in the tissues.

The vessels of the *spleen* were enormously dilated and there were numerous large and small hæmorrhages in the splenic pulp. Within

the blood-vessels and in the hæmorrhagic areas bacilli in large and small clumps or masses were seen. They never completely plugged the capillaries. In the veins and occasionally in the capillaries liver cells singly and in short rows were found; these nowhere, however, seemed to fill the lumina of the vessels. Lying free in the tissue spaces and often inside of cells, both polymorphonuclear leucocytes and lymphocytes, were larger and smaller yellow pigment masses, which appeared not only as fine granules, but also in large irregular masses. This pigment stained deeply with eosin and with picric acid. There was no special hyperplasia of the splenic tissue, but the polymorphonuclear leucocytes were increased in number, both in the blood-vessels and in the tissues. There were definite areas of tissue necrosis. The red corpuscles both in the vessels and in the areas of hæmorrhage were pale and stained poorly.

Kidneys.—Fresh frozen sections of the kidneys showed cloudy swelling and fatty degeneration of the epithelial cells of the convoluted tubules and of the glomerular epithelium. In sections hardened in Flemming's solution fat drops were noted in the endothelial cells of the glomerular capillaries. The blood-vessels, both large and small, in all parts of the organs were greatly distended with blood. There was a notable increase of the large mononuclear as well as of the polymorphonuclear leucocytes. In many of the veins liver cells were present without occluding completely the lumen. The glomerular capillaries were widely dilated and contained an increased number of cells. The glomeruli were swollen and completely filled their capsules. The epithelial cells of the convoluted tubules were swollen, very granular and often devoid of nuclei; some were desquamated. There was no hæmorrhage into the glomerular capsules or into the tubules. There were a few small areas of round-celled infiltration with atrophy of the renal tissue, and a slight amount of arterio-sclerosis of the smaller vessels. Except in the large blood-vessels, few bacilli were to be found in the sections of the kidney. None were seen free in the tissues.

In the *stomach* and *intestine* there was marked dilatation of the vessels, especially of the serous, mucous and submucous coats. In the ileum there was hyperplasia of Peyer's patches and of the diffusely spread lymphoid tissue. The mesenteric lymphatic glands showed congestion and histological changes similar to those in the spleen.

The *thyroid gland* showed marked hypertrophy, the veins and capillaries being very much dilated. Bacilli were found in the vessels.

Bacteriological examination.—The bacterial forms found were the same in all the organs, and were most numerous in the blood-vessels, especially the veins. Only in the liver were bacilli found outside the

vessels. They were demonstrated best in sections stained first with lithium carmin and then with Weigert's fibrin stain. They varied much in size and in shape. Rod-shaped forms were the most common but occasionally forms suggesting large cocci and diplococci were found. Many short and long, oval forms were seen. Some of the rods were short and stout with rounded ends; others longer (1 to 2 μ), while some were long filaments (3 to 5 μ). Sometimes two or more rods were seen end-to-end, while others did not stain throughout but presented a beaded appearance. Capsulated forms were occasionally seen.

At the time of the autopsy coverslip preparations were made, as well as cultures in Esmarch roll-tubes in agar-agar, from the blood of the heart, from the lungs, liver, kidneys, spleen, a mesenteric lymph gland and the brain.

The examination of coverslips from several of these (heart's blood, liver and spleen) showed bacilli and oval forms, usually surrounded by a capsule, similar to those described in the hardened sections. All the cultures from the blood and various organs, after incubation for 18 hours, showed a luxuriant growth of colonies which had the same appearance in all the tubes. The same organism was found in pure culture in all the tubes.

Agar, plain or with glycerine or glucose. In cultures made in Esmarch tubes or in Petri dishes, after 18 to 24 hours in the incubator at 37° C., small, deep and larger superficial colonies appear. The larger colonies are from 1 to 2 or 3 mm. in diameter, raised above the surface of the medium, usually round in outline, and tending to pile up in the centre. They are thick, opaque, have a grayish white appearance, and tend to spread. Magnified 50 diameters the colonies are dark brownish yellow in the centre, and of a homogeneous appearance. The edges are lighter brown and somewhat irregular in outline. The deeper colonies are grayish white, opaque, and usually small. Those that reach the surface tend to pile up without spreading. Microscopically they are round, with clear-cut borders, dark brown in color and finely granular. After a few days these colonies, under the microscope by transmitted light, are darker, almost black in color, homogeneous, and somewhat oblong in shape. Streak cultures upon slants of plain agar show in 24 hours in the incubator and 48 hours at room temperature an abundant, luxuriant raised growth along the line of the needle. This growth is creamy white, polished, porcelain-like, standing well up above the surface of the medium, and usually with serrated edges. In a few days the growth spreads over the surface of the medium. The growth on glycerine agar and glucose agar has the same characters, but

is more luxuriant. Stab cultures in plain nutrient agar show after 18 to 24 hours in the incubator a feeble, white growth along the line of stab. Similar cultures in glycerine agar and glucose agar grow luxuriantly with the production in the latter of a large amount of gas. In old agar cultures the medium becomes brownish and there is an odor like that of stale glue. The growth is always viscid and sticky; gas bubbles are frequently seen in the water of condensation from glucose agar.

Blood serum.—The colonies on solidified blood serum are quite similar to those on agar. The growth is rapid and luxuriant, viscid and mucoid, pale, grayish-white, porcelain-like, raised, and tending to spread from the line of the inoculation. In a few days it covers nearly the whole of the surface. The water of condensation is diffusely clouded: the medium is not liquefied.

Gelatine.—At room temperature after 36 hours in plate or roll cultures there appear small, round, opaque, creamy white colonies 0.5 mm. in diameter. The centre of the colony is thicker and more elevated. Magnified 50 times, the colonies are of a yellowish color by transmitted light. Some of them tend to spread, but the majority have regular outlines. In some of the larger colonies the centre is umbilicated, while the peripheral portion is raised and arranged in concentric layers. The deep colonies are round with clear-cut margins, and of a dark brown almost black color when examined under the lower powers. The larger colonies when magnified 50 diameters are dark brown in the centre, fading to a dark brownish yellow homogeneous appearance at the periphery. The edges are light brown in color and rather irregular. Gelatine slant cultures after 24 hours at room temperature show a raised growth similar to that seen on agar and on blood serum, but less luxuriant. In gelatine stab cultures, along the line of the stab after 24 hours there appear small scattered grayish white colonies. At the surface of the culture is a small, raised, opaque, grayish white, round or oval growth, which after a few days tends to spread out over the surrounding surface. No well-marked, nail-shaped growth developed. Gelatine is not liquefied. In old cultures the medium sometimes becomes brownish. Gas production does not occur in plain nutrient gelatine.

Potato.—The bacillus grows luxuriantly upon potato both at room and body temperatures. In test-tube potato cultures the upper third of the growth does not usually spread from the line of inoculation, and is generally grayish white, sometimes dark brownish gray, dry and finely granular. The lower two-thirds of the culture nearly always spreads, so that here the whole surface and often the sides of the medium are

covered with a moist growth. This growth is sometimes raised and porcelain-like, and of a creamy color, sometimes simply smooth, moist and polished, without piling up. There is always gas production both upon the surface of the potato and in the fluid at the bottom of the tube.

Carrot.—The growth on this medium is like that on potato, but more luxuriant and with more marked gas formation.

Bouillon.—The growth in plain as well as in glucose and glycerine bouillon is rapid and luxuriant both at room temperature and in the incubator. After 24 hours' incubation the medium is diffusely cloudy. On the surface is a grayish white pellicle, adherent to the sides of the tube and usually covering the whole of the surface. At the bottom is an abundant, grayish white sediment, which on agitation breaks up into a thick stringy mass. In cultures several days old the medium becomes almost opaque, thick, tenacious and stringy like mucus. In bouillon containing glucose, saccharose, or any of the sugars there is abundant gas production. In the fermentation tube within 24 hours sufficient gas is formed from glucose bouillon to displace nearly all the medium in the upright arm.

Milk.—Milk is coagulated with gas formation and acid production, in from 24 to 36 hours in the incubator. The coagulum is hard and forms a solid mass surrounded by thin pale whey.

Dunham's peptone solution.—The bacillus grows rapidly in this medium and with the same characters as in bouillon. No indol reaction could be obtained.

The organism grows best in media of neutral or alkaline reaction, but will grow also on acid gelatine, potato and carrot. It grows best at 35°-37° C. It is killed by exposure for five minutes to a temperature of 60° C. It is non-motile and does not form spores. It is facultatively anaërobic, and stains well by both Gram's and Weigert's methods, as well as with the common aniline dyes.

General morphology of the bacillus. The morphological appearances of the bacillus are the same in the *blood* and *organs* of animals as in those of the human case. Coverslips made from the liver, spleen, kidneys and heart's blood showed bacilli often in pairs, varying from 0.5 to 3 or 4 μ in length, by 0.5 to 0.8 μ in thickness, generally with rounded ends. There were marked variations in size and shape. Many short oval forms were seen. Short forms resembling cocci and diplococci occurred, and occasionally long filaments were seen. The long forms often had square ends, while the shorter ones always had rounded ends or were ovoid in shape. Coverslips from the fresh blood or from the

organs always showed capsulated forms, the capsules frequently containing pairs. The shorter organisms were encapsulated more frequently than the larger ones. From the pleural and peritoneal exudates the organisms constantly showed capsules. Here the coccal, oval and short bacillary forms are the most common, long rods being unusual.

The capsules stain readily with carbolic fuchsin, with eosin and gentian violet, with or without aniline oil. The organism stains readily with all the usual dyes, and can be seen even in sections stained with eosin and hæmatoxylin. As previously stated, capsulated forms are rarely seen in sections of hardened tissues. In cultures there is the same polymorphism as that found in the human body. In fresh agar, glycerine agar, blood serum and gelatine cultures the most numerous forms are short, plump bacilli, with rounded ends; fewer coccal forms are seen, but there are often ovoid forms in pairs or short chains; some long stout bacilli are also seen. In young cultures, especially on glycerine-agar and on blood serum, capsulated forms are often seen. In fluid media the longer forms predominate, and many long filaments of from 5 to 8 or even 10 μ in length are seen. In older cultures on solid media the longer forms are numerous, and sometimes there are irregular swellings. On old potato or carrot cultures the predominating forms are long. Most of the longer forms stain poorly, and in many there are one, two, or even three deeply staining points, surrounded by somewhat granular, poorly staining protoplasm. In some there is an exquisite bipolar staining, while in others only one pole takes up the dye. Nothing suggestive of spores is to be seen.

Pathogenesis.—Cultures of the bacillus, both fresh and old, grown both on solid and in fluid media, both living and sterilized, were pathogenic for dogs, rabbits, guinea-pigs, white and gray rats, and house mice, while pigeons proved refractory.

Dogs.—Young dogs inoculated into the peritoneal cavity with 2 cc. of a 24-hour-old bouillon culture died in 36 hours with a thick purulent exudation in the peritoneal cavity, with marked congestion and ecchymoses of the peritoneum and congestion of the pericardium, pleuræ, liver, spleen, kidneys, stomach and intestine.

Rabbits.—Many rabbits were inoculated in the ear vein, subcutaneously, and into the peritoneal and pleural cavities, in doses varying from 0.2 cc. to 3 cc. of a 24-hour bouillon culture, or of a suspension in bouillon or salt solution of several loops of a blood-serum or agar culture. The smaller doses without exception were fatal, even for old, large animals, except when inoculated subcutaneously.

Intravenous inoculations killed rabbits in from 12 to 72 hours, the

animals dying of septicæmia with congestion of the various organs and serous membranes, with hæmorrhages in many instances, and sometimes with fibrino-purulent exudations. The spleen was not enlarged, and pneumonia was never found. Fatty degeneration of the liver, heart, and kidneys was commonly present.

Peritoneal inoculations killed in from 12 to 72 hours by septicæmia and peritonitis, often with hæmorrhages, and the formation of a stringy, mucoid exudate rich in short bacilli, both free and within polymorphonuclear leucocytes. On microscopical examination the veins and capillaries were found widely dilated, especially in the stomach, intestine, liver and kidneys. There were no focal areas of cell degeneration in any of the organs. The bacilli were abundant in the blood and organs.

Subcutaneous inoculations caused local purulent exudates, often followed by death from septicæmia with changes in the organs and vessels similar to those described above.

White *rats* died in from 24 to 48 hours after subcutaneous or intraperitoneal inoculation of 0.5 to 1 cc. of a bouillon culture. The organisms were always present in the heart's blood, in the various organs and in the peritoneal and pleural cavities. The same dilatation of the blood-vessels with focal hæmorrhages, as has been described in rabbits, was observed in these animals.

Wild gray rats died in from 18 to 24 hours, after either subcutaneous or intraperitoneal injection of small doses, with septicæmia and few changes in the organs.

House *mice* died on the fourth day after inoculation, whatever the mode of inoculation. There was seldom any local reaction at the point of inoculation, but septicæmia, with marked congestion of the vessels and multiple ecchymoses of the serous membranes and various organs, was present. The serous cavities contained an excess of fluid, which was thick, tenacious and stringy, and often contained polymorphonuclear leucocytes, endothelial cells, and red corpuscles, in addition to large numbers of bacilli. In the liver the most striking change was the enormous dilatation of veins and capillaries. The veins of both the portal and hepatic systems, particularly just beneath the capsule, were distended with blood and the liver-cells were compressed and granular, and often contained large and small fat drops. Here and there were larger and smaller points of hæmorrhage. Many bacilli were seen in the capillaries and in the hæmorrhagic areas. Emboli of bacilli, however, were never seen. The lungs showed marked dilatation of the blood-vessels, in which bacteria, singly and in clumps, were present. Besides these, some unmistakable liver cells, granular and fatty, were detected in

the pulmonary arteries and capillaries. In the spleen, especially under the capsule, there were marked congestion and often hæmorrhage into the tissue. The red corpuscles in the tissue were in various stages of degeneration, and a large amount of yellow pigment was seen. In the kidneys the most marked change was hyperæmia, with here and there areas of hæmorrhage into the tubules and inter-tubular tissue. Bacilli were in the blood-vessels in large numbers, especially in the glomerular capillaries. They also appeared in masses in the tubules. The epithelium of the convoluted tubules was fatty. There were no focal areas of degeneration or of round-celled infiltration. In all the coats of the stomach and intestine there were congestion and hæmorrhages.

Guinea-pigs.—Peritoneal inoculations of from 0.2 to 0.5 cc. of a 24-hour-old bouillon culture caused death in from 12 to 48 hours. The inguinal and axillary glands were swollen and congested, with œdema of the periglandular tissue. There was always a peritoneal exudate, which in rapidly fatal cases was serous with few cells, and in animals living longer, thick and tenacious, with grayish opaque patches, containing many endothelial and polymorphonuclear cells. There was always congestion of and often hæmorrhages into the serous membranes. The heart was pale; the liver enlarged and deeply congested. Microscopically the same changes were found in the organs as in mice and rabbits. These were especially marked in the liver, where the central veins were often ruptured. The blood-vessels contained a large number of polymorphonuclear leucocytes and many bacteria, some of the capillaries being plugged with the latter. Here and there clumps of bacilli, surrounded by polymorphonuclear leucocytes, were seen in the tissues.

Subcutaneous inoculations in guinea-pigs were followed by abscess formation, œdema, and death from septicæmia after from 3 to 6 days; the autopsy showed the vascular and tissue lesions as in animals inoculated in other ways.

Pigeons were refractory to large doses administered either subcutaneously or peritoneally.

Bouillon cultures from two to six weeks old, sterilized by heat or by the addition of 0.5 per cent. trikresol, in doses from 0.25 to 0.5 cc. and over, killed rabbits, guinea-pigs and white rats; they were not pathogenic for dogs, but even in minute doses killed house mice. The bacteria-free filtrate of such cultures was also pathogenic for rabbits, guinea-pigs, and house mice in the same doses as the unfiltered cultures. The dead bacteria left in the filter even after repeated washings with water were pathogenic for the same animals.

An analysis of the results of experiments upon a large number of animals shows that the sterilized unfiltered cultures were the most toxic, the dead and washed bacteria stood next in toxicity, while the filtrate of the same cultures was least toxic. The animals inoculated into the ear vein or intraperitoneally with these substances became ill and died in from 6 to 18 hours. They showed marked congestion of the serous and mucous membranes with ecchymoses, congestion and hæmorrhage into the various organs. The liver cells and the epithelial cells of the kidney showed cloudy swelling and fatty degeneration, but there were no large areas of focal necrosis. The blood-vessels were widely distended, and there were marked hæmorrhages in the liver, spleen and kidneys. In the spleen there was found a large amount of yellow pigment, both intra- and extra-cellular. The pigment was usually in the shape of small granules, but larger masses were seen.

By starting with small non-fatal doses of both living and sterilized cultures and the filtrates of bouillon cultures, both guinea-pigs and rabbits could be accustomed to withstand doses fatal for untreated animals (1 to 1.5 cc.); but for these animals a dose of 2 cc. was fatal.

The blood serum of rabbits or guinea-pigs thus immunized from dead cultures and soluble toxines, even in large amounts (0.5 to 1 cc.), failed to protect fresh animals against the toxines or the dead or living cultures.

One large pregnant rabbit, receiving first small doses and then as much as 1.5 cc. of a sterilized bouillon culture on two occasions, carried her young to term. The mother, after gestation, became thin and her young were much smaller and grew much less rapidly than those of other rabbits born about the same time. Two of her young rabbits, when six weeks old, were inoculated with 0.5 cc. of a 24-hour bouillon culture of the bacillus, a fatal dose for a control rabbit of the same age but out of another mother. One of the two rabbits died within 24 hours; the other was apparently unaffected. 0.5 cc. of the blood serum of a third rabbit of this litter failed to protect one of another litter against a similar quantity of a 24-hour bouillon culture of the bacillus, but a large rabbit receiving 1 cc. of the same blood serum and 1 cc. of a sterilized bouillon culture of the bacillus did not die till the fifth day. From this it would seem probable that rabbits may inherit from the mother some degree of tolerance for this organism.

The bacillus above described has preserved its virulence and general biological characters during the five years since it was obtained

at autopsy. When compared by means of cultures and animal experiments with a number of capsulated bacilli of the type of Bacillus pneumoniæ of Friedländer,—including two examples of the bacillus of Friedländer obtained from cases of pneumonia, the capsulated bacillus of Pfeiffer, the bacillus of Wright and Mallory, and a capsulated bacillus obtained by Dr. Walter Reed, U. S. A., from the throat in a case of diphtheria, besides a number of capsulated bacilli obtained by the writer from inflammatory processes of the accessory sinuses of the nose, and from two cases of septicæmia, one following labor and the other pyelonephritis—the bacillus of the present case showed differences from them all. Of these differences the chief related to the varying amounts of gas and acid production, the capability and the rapidity of coagulation of milk, the behavior to Gram's stain, and the widely varying results of animal inoculation.

The bacilli above mentioned, as well as other allied forms, have been placed by Fricke (13) in a group of which the Friedländer bacillus is the prototype and to which E. Fraenkel gave the name "Bacillus mucosus capsulatus." The more important common characteristics of this group are the morphology—plump, medium-sized, pleomorphic rods; the presence of capsules, readily demonstrable in the animal body and sometimes in cultures; lack of motility and of spores; failure as a rule to stain by Gram; the rapid, luxuriant, elevated, viscid, white growth upon the surface of solid media; absence of liquefaction of gelatine; and pathogenicity, usually in the form of septicæmia, but with striking variations for different animals and for different members of the group. Fricke has collected from the literature 22 representatives of this group and to these has added from personal observations still others, which differ from each other more or less sharply by such points as minor cultural variations, particularly on potato, presence or absence of brownish discoloration of old gelatine or agar cultures and of a typical "nail-growth," the coagulation of milk, the occurrence of indol, production of gas and of acid, retention of Gram's stain, and, above all, susceptibility of different animals under varying methods of inoculation to the pathogenic effects of the organism. Opinion is divided, and it is at present indeed difficult to

decide, as to the value of these several points in the establishment of species or varieties. They have not hitherto sufficed for any satisfactory subdivision of the group into well-recognized species or varieties, although attempts to accomplish this have been made.

While the bacillus described in this paper cannot be identified with the bacillus of Friedländer and differs in certain respects from most, if not all, previously described members of the group, it nevertheless possesses the more important common characteristics of the group, and I therefore prefer, at least for the present, to place it here rather than to propose a new group. It is true that Kruse * emphasizes the absence of staining by Gram as one of the characters of his group of Bacillus aërogenes and bacillus of rhinoscleroma, under which group he classifies the various capsulated bacilli of the type of the bacillus of Friedländer, but the readiness with which the color can be extracted by Gram's method is certainly one of the variable attributes of the group and cannot serve as a decisive criterion in determining whether an organism belongs to the group or not.

Doubtless to the same group of capsulated bacilli (Bacillus mucosus capsulatus) belong bacilli isolated from human cases of hæmorrhagic infections by Bordoni-Uffreduzzi (6), Banti (5), Babès and Opresen (4), and von Dungern (10). We may, therefore, consider it established that members of this group of capsulated bacilli may cause typical hæmorrhagic septicæmia in man.

Different from the preceding are the bacilli cultivated from cases of septic purpura hæmorrhagica by Babès (1), Tizzoni and Giovannini (22), and Kolb (17). Babès classifies these bacilli together, although it is not certain that they are closely allied, and he considers that they have many points in common with Hueppe's hæmorrhagic septicæmia group. Their resemblance to Hueppe's group seems to me rather remote, but it must suffice here to call attention to these bacilli as distinct from the luxuriantly growing, capsulated bacilli already described.

It is clear that various bacteria, not only those already considered, but also streptococci and other bacteria, may cause hæmorrhagic infec-

* Flügge's Die Mikroorganismen, Th. II, p. 336; Leipzig, 1896.

tions in human beings. Haemorrhagic septicaemia, then, is not to be regarded as a separate and distinct disease, with definite and constant etiology, uniform anatomical lesions and clinical features, as, for instance, typhoid fever, but rather as a toxaemia, attended by such marked injury to the blood-vessels and to the blood as to cause extreme dilatation and hæmorrhages, with various harmful effects upon the body cells, and capable of being caused by a variety of agents. The organic lesions differ widely in various cases. The similarity in the effects produced in animals and in man by the bacillus of my case is striking. In both, beside the vascular changes, fatty degeneration of the endothelial cells, and rupture with hæmorrhage into the tissues, there was marked destruction of the red corpuscles, with the accumulation of blood pigment in the liver, spleen and kidneys. Of interest also in my case is the direct and the retrograde embolism of the liver cells. The similarity of my cases to typhus fever is paralleled by the case reported by Babès and Oprescu. A malignant, rapidly fatal course has characterized several of the reported cases, whereas others have been of milder type and longer duration. At least some of the instances reported as malignant purpura hæmorrhagica belong to the class of hæmorrhagic septicæmias.

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BACILLUS CAPSULATUS (BACILLUS PNEUMONIÆ OF FRIEDLAENDER?) WITH ESPECIAL REFERENCE TO ITS CONNECTION WITH ACUTE LOBAR PNEUMONIA.

A Report of Twelve Cases in which Bacillus Capsulatus Occurred in the Medical and Surgical Wards of the Boston City Hospital.

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Method. The method of investigation will be further described with the reports of cases. Löffler's blood serum slants were used as a routine procedure. Descriptions of growth, appearances of colonies, etc., refer to this medium unless otherwise mentioned. In addition to the blood serum the various solid and fluid media were used, as well as animal inoculations and various special staining methods.

REPORTS OF CASES.

The twelve cases in which the Bacillus capsulatus occurred were as follows: (These cases, with the one reported by Wright and Mallory (1) in 1895, represent all the cases in which the capsulated bacillus was observed in the Boston City Hospital during the past three years.)

Case I. Acute lobar pneumonia. The patient entered the hospital March 15, 1896, in the service of Dr. V. Y. Bowditch, with considerable dyspnœa and cyanosis and with signs of consolidation in the middle lobe of the right lung. Death occurred within 24 hours after admission.

Autopsy (Dr. Mallory), March 16. *Anatomical diagnosis: Acute fibrinous pneumonia of middle lobe of right lung, with extension into upper and lower lobes. Cloudy swelling of liver, kidneys and heart, and acute splenic tumor.*

Coverslips from consolidated lung at autopsy showed numerous large and small bacilli with rounded ends surrounded by a wide capsule, also many small lanceolate, encapsulated diplococci. The bacilli were completely decolorized by Gram, whereas the lanceolate cocci retained the stain.

Cultures from the hepatized right lung showed a profuse, confluent, colorless, stringy, mucoid growth with a few small, slightly opaque, pinhead colonies at the top of the culture medium. The profuse growth contained mostly large bacilli with rounded ends, surrounded by wide capsules containing from one to four rods, decolorizing slowly by Gram. The small colonies consisted of irregular groups of small cocci. The water of condensation contained both the large capsulated bacilli and many lanceolate diplococci.

From the liver there grew a number of elevated viscid colonies, varying in size from a pin's head to 5 mm. in diameter. These showed the same capsulated bacillus as in the lung.

The culture from the kidney contained 8 to 10 colonies similar to those from the liver. Cultures from the heart and spleen were negative.

Microscopical sections of the middle lobe showed distended alveoli filled with fibrin, red corpuscles, leucocytes and a moderate number of desquamated cells. By the Gram-Weigert stain many lancet-shaped diplococci were found in the alveolar and pleural exudates. In sections stained by Unna's alkaline methylene-blue were seen a few large and medium-sized bacilli in the alveolar exudate, and large numbers in the bronchi. In sections stained by the Gram-Weigert method no bacilli were observed.

The capsulated bacillus was completely decolorized in smears from the lung, in preparations from cultures, and in sections stained by the Gram-Weigert method, the decolorization in smears being considerably slower than in preparations from cultures.

Case II. Gangrene of the lung. Patient aged 68 years, a roofer by trade, was brought to the hospital in a moribund condition. From patient's brother it was learned that he had been sick for four weeks; there was considerable expectoration emitting a foul odor, and of a purulent character, sometimes containing blood. Physical examination showed evidences of a large cavity in an area about 2½ inches in diameter just below the right clavicle. Patient died on the day following admission.

Autopsy by Dr. Mallory. Anatomical diagnosis: *General arteriosclerosis; gangrene of upper lobe of right lung, and acute vegetative endocarditis.*

Upon the mitral valve were fresh vegetations. In the right lung was a large cavity occupying almost the entire upper lobe, filled with a foul-smelling fluid, which contained small pieces of detached gangrenous lung. Other parts of this lung and the entire left lung were normal.

Coverslips from material in the pulmonary cavity showed many large and small capsulated bacilli, some cocci and a few slightly staining rods. Coverslips from the mitral vegetations showed a few medium-sized capsulated bacilli which decolorized by Gram's method.

Cultures from the upper lobe of the right lung presented a diffuse, translucent, stringy, mucoid growth of numerous small and large bacilli, some faintly staining rods without capsules, and a small number of cocci.

From the heart grew 10 to 12 viscid colonies, varying in size from a pin's head to 4 mm. in diameter, similar to those described in Case I.

The tube from the spleen showed 5 colonies, similar to those from the heart, and containing the same capsulated bacillus. Cultures from the liver and kidneys were sterile.

All the large colonies were composed of the capsulated bacillus. A guinea-pig, weighing 410 grms., inoculated in the peritoneal cavity with a pure culture on blood serum, died in 17 hours with general peritonitis and marked congestion of the liver and spleen. Cultures from the animal gave a profuse growth of the same organism as that from Case I.

Case III. Acute croupous pneumonia complicated with acute otitis media. The Bacillus capsulatus and the diphtheria bacillus were found in cultures made from the middle ear. The Micrococcus lanceolatus was found in the lung, heart's blood and kidney, but not in the middle ear in which only the capsulated bacillus and the diphtheria bacillus were demonstrated. There was no extension of inflammation from the ear into the mastoid sinuses or the cranial cavity.

Case IV. Fracture of base of skull accompanied by acute otitis media. The capsulated bacillus was found in pure culture in the middle ear, from which there was no extension of inflammation.

Case V. Diphtheria. In a young man, about 21, convalescing from diphtheria, the capsulated bacillus was found, with the diphtheria bacillus, in cultures from the throat. The capsulated bacillus persisted in daily cultures from the throat, until the patient was discharged nearly five weeks later. Diphtheria bacilli were present in small numbers for four weeks. Pure cultures of the diphtheria bacillus, isolated during four weeks, failed to kill guinea-pigs, even when injected in large quantities.

Case VI. After diphtheria. Culture from the throat showed diffuse growth of capsulated bacilli with a few streptococci, but no diphtheria bacilli.

Case VII. Diphtheria. Capsulated bacilli and diphtheria bacilli isolated in pure cultures. In the nose there were no capsulated bacilli

but many diphtheria bacilli. In the culture from the throat the capsulated bacilli grew so profusely that the diphtheria colonies could not be made out. The diphtheria bacilli in small numbers were seen only after smearing from the general surface of the serum tube in the water of condensation. The growth of the capsulated bacilli disappeared in three or four days, after which the diphtheria colonies grew luxuriantly.

Case VIII. Diphtheria. A few colonies of diphtheria bacilli and many large colonies of the capsulated bacillus.

Case IX. Tonsillitis and pharyngitis. Cultures showed a profuse growth of the capsulated bacillus and in the water of condensation a moderate number of streptococci. Patient ill three days.

Case X. Diphtheria. Cultures from the throat gave a profuse growth of capsulated bacilli and a few colonies of diphtheria bacilli. The cultures were examined daily for two weeks. The capsulated bacillus persisted up to the time of discharge. The diphtheria bacillus disappeared in a little over one week. The attack was very mild. The mucous membrane of the pharynx was congested, granular, and covered with a thick, glairy, slightly opaque, sticky material, resembling a diffuse growth of the capsulated bacillus on Löffler's blood serum. This material when touched with a platinum loop would draw out in long threads.

Case XI. Tonsillitis. Cultures from the throat gave a profuse growth of capsulated bacilli with a few chains of streptococci. The symptoms cleared up in a few days.

Case XII. Diphtheria. Cultures from the throat showed many capsulated bacilli and a few diphtheria bacilli.

Three other of the throat cases were inspected clinically by the writer and the throats showed the presence of a mucoid, glairy material similar to that in Case X. Of the foregoing twelve cases, the first four were seen by the writer only at the post-mortem examination. The other cases were observed clinically as well as studied bacteriologically. The six diphtheria cases were mild. The two instances of tonsillitis presented no unusual symptoms and recovered after a few days. In these eight cases, therefore, the severity of the disease was not increased by the presence of the capsulated bacillus.

In Case I the capsulated bacillus was found plentifully in the bronchi, while but few were present in the alveolar exudate. It evidently was not concerned in the pneumonic process. An acute endo-

carditis caused by the capsulated bacillus, as in Case II, is rare. The writer has been unable to find a report of a similar case in this country. Weichselbaum (2) in 1888 reported an instance of acute endocarditis due to a similar capsulated bacillus which he called Bacillus capsulatus endocarditidis.

In Case IV, in which the capsulated bacillus was found in pure culture in the middle ear there was no extension into the mastoid cells or the cranial cavity. Reports of cases of acute otitis media due to capsulated bacilli, while not uncommon abroad, especially in Germany, are not very frequent in this country. In Case III the capsulated bacillus was found with the diphtheria bacillus, and it is a question whether the former was responsible for the inflammation, as we have observed several cases of acute otitis media in this hospital due to the Klebs-Löffler bacillus alone.

It is evident from these cases that the Bacillus capsulatus, while not common in this country, is, nevertheless, not an extremely rare organism. Only in two cases (II and IV), did it seem to have any special pathological significance. Of the other two cases in which the bacillus was found at autopsy, in Case I there was a double infection and death was due to the pneumonia which was caused by the Micrococcus lanceolatus, and in Case III, death resulted from pneumonia and general infection with the Micrococcus lanceolatus.

DESCRIPTION OF THE BACILLUS.

The following description of the capsulated bacillus obtained from the lung of Case I answers for each of the twelve cases.

The bacillus is thick, of variable size, with rounded ends, on an average from two to three times as long as broad, enclosed in an oval wide capsule, and often united in rows of two, three or four elements within a single capsule. Sometimes it grows out into rods five or six times as long as broad. It stains with the usual aniline colors, but not by Gram, by which it is slowly decolorized. The capsule is constant both in tissues and in cultures. The capsule can be stained with any of the usual dyes when dilute acetic acid is used for washing out the excess of stain. The capsule is best demonstrated by the methods described under *capsule stain* (p. 175).

The capsulated bacilli isolated from the twelve cases are essentially identical. There are slight differences only in degrees of virulence, when inoculated subcutaneously into guinea-pigs. This bacillus is practically identical with that described by Wright and Mallory (1), the main difference being that Wright and Mallory's bacillus did not kill guinea-pigs by subcutaneous inoculatien, whereas subcutaneous inoculation of guinea-pigs with our bacillus was fatal in from five to seven days, and after death the bacilli were found in each case in the heart's blood and the various organs.

Colonies on blood serum after 18 hours appear as slimy drops, transparent, round, elevated, with convex surface. The size varies from a pin's head up to 5 mm. in diameter. A confluent growth covering the entire surface of the medium is often seen. The colonies are thick and stringy, and when touched with a platinum loop draw out in long threads. The water of condensation is thick and of a whitish color. On 1 per cent. glucose agar slants the growth appears as a broad viscid transparent line. The water of condensation is thickened. Stab cultures in 1 per cent. glucose agar show gas formation at the bottom of the tube, and growth along the entire line of inoculation. In gelatine stabs, growth occurs along the entire needle track. At the point of inoculation there is an elevated mound-like knob or nail-head which is like that described by Friedländer (3). Bouillon becomes cloudy after 15 hours. The bouillon is thickened and viscid at the end of 24 hours. On potato there is a profuse, glairy, colorless, viscid growth. Milk is coagulated and acidified.

The organism kills white mice and rabbits when inoculated into the ear vein. In guinea-pigs intra-peritoneal injections kill in 24 hours, subcutaneous inoculation in from 5 to 7 days. At the autopsy there are found enlargement of lymph glands, a large soft spleen, the blood somewhat thickened, but not to the degree described by Pfeiffer (4) in his experiments with the capsulated bacillus. The adrenal glands of the guinea-pigs were hæmorrhagic as in the experiments of Wright and Mallory.

The organism here described is closely related to, if not identical with, the bacillus of Friedländer (3), and its description agrees closely

with that of the capsulated bacilli described by Wright and Mallory (1). Pfeiffer (4), Fasching (5), von Dungern (6), Mori (7), Mandry (8), Abel (9), Paulsen (10), Marchand (11). Loeb (12) and others have described capsulated bacilli differing from ours only in minor details and many of them are doubtless identical. Most of the studies of this group of bacilli have been made by foreign investigators. Of the varieties which most closely resemble ours, besides that of Wright and Mallory, may be mentioned those of Friedländer, of Pfeiffer, of Fasching and of Loeb. It is also probable that many described by others are identical and represent varieties of varying virulence. They certainly are all closely related.

Several attempts have been made to classify the various capsulated bacilli but without any great success. Wilde (13) has attempted to divide them into five groups, but his classification can scarcely be recommended. Fricke (14) has made a careful comparative study of the members of this group and has collated the characters of many reported in the literature.

Capsule stain. For staining the capsules both in cultures and in cover-glass preparations made from the organs a modification of Welch's method was used.

Welch's (15) method is as follows:

1. Cover the preparation (prepared without contact with water) with glacial acetic acid for a few seconds.

2. Drain off and replace (without washing in water) with aniline-gentian-violet solution. The staining solution is to be repeatedly added to the surface of the cover-glass until all of the acid is replaced.

3. Wash in aqueous solution of sodium chloride and examine in the same. The strength of the salt solution varies in different cases from 0.5 to 2 per cent.

This method depends upon the precipitation of the mucin-like substance of which the capsule is composed by the acetic acid, the precipitated material being insoluble in a 2 per cent. or sometimes weaker solution of sodium chloride.

I have found that by this method after using the salt solution, the specimen was often covered by a granular deeply staining detritus which often made it difficult to differentiate the capsule. The following modification was used by me and found satisfactory. It gives a much clearer picture and the capsules are stained more deeply.

1. Cover the preparation with glacial acetic acid for a few seconds.

2. Wash off the acetic acid with a 1 per cent. solution of potassium hydroxide.

3. Stain with aniline-gentian-violet for one minute without previously washing off the potassium solution.

4. Wash off excess of stain quickly in water.

5. Dry thoroughly with filter paper and over low flame and mount in balsam.

If the specimen is stained too deeply it may be decolorized by washing lightly in a 0.5 per cent. solution of acetic acid. The specimen should be completely dried before mounting in balsam, otherwise the bacilli will soon decolorize. This method is also well adapted for staining the capsules of the Micrococcus lanceolatus. I have coverslips prepared in this way which have not faded after two years. It may be added that while it is very easy to stain the capsules of the bacillus, it is often very difficult to stain those of the Micrococcus lanceolatus. If the slightest amount of water touches the specimen before the acetic acid is used the capsules are not stained but appear as clear halos around the cocci.

For staining the bacilli in sections Unna's strong alkaline methylene-blue, after the manner described by Mallory and Wright (16), was used. This stain is the most satisfactory to use for bacteria which decolorize by Gram and in connection with the Gram stain. Sections of lung from Case I showed very well the comparative numbers of bacilli and cocci.

RELATION OF THE CAPSULATED BACILLUS TO ACUTE LOBAR PNEUMONIA.

The history of the discoveries concerning the presence of bacteria in croupous pneumonia from the first observations of Klebs (17) in 1875 to the decisive papers of A. Fraenkel (18) and of Weichselbaum (19) in 1886 has been fully given by Welch (15) and need not be here repeated. Fraenkel came to the conclusion that the Micrococcus lanceolatus is the sole cause of genuine acute lobar pneumonia, whereas Weichselbaum, while recognizing this organism as the principal cause, claimed that about 5.5 per cent. of the cases of typical croupous pneumonia are referable to the Bacillus pneumoniæ of Friedländer. While the majority of investigators are probably of Fraenkel's opinion, not a few, especially the writers of text-books, hold Weichselbaum's view that a small percentage of cases of acute lobar pneumonia may be caused by the Friedländer bacillus and even

by other bacteria. Finkler (20), Houl (21) and Ziegler (22) may be cited as advocates of the latter view.

It has been our experience in the Boston City Hospital to find that true acute lobar pneumonia is invariably due to the Micrococcus lanceolatus. Pearce (23) in his report of 121 cases of acute lobar pneumonia which came to autopsy in this hospital from May, 1894, to May, 1897, found the Micrococcus lanceolatus in 118 or in 97½ per cent. of the entire number. The writer (24) reported in April, 1896, before the Boston City Hospital Medical Society, his investigations of 32 consecutive cases of acute lobar pneumonia. In every one the Micrococcus lanceolatus was found. Welch (15) found it in 10 consecutive cases at the Johns Hopkins Pathological Laboratory. Like results have been reported by many other observers.

It is an easy matter to overlook the Micrococcus lanceolatus, especially in mixed infections, as is illustrated by our Case I. In this case the cultures from the consolidated lung showed apparently on first inspection only the capsulated bacillus, but examination of sections stained by Gram-Weigert and careful examination of cultures with the application of the Gram stain revealed the presence of the Micrococcus lanceolatus. Undoubtedly many of Friedländer's cases were double infections, as he himself described the micrococci in the alveolar exudate and obtained the capsulated bacillus in cultures. It is of interest that Gram (25), working under Friedländer's direction, devised his stain for the purpose of demonstrating organisms in croupous pneumonia which he at the time believed to be identical with the capsulated bacteria obtained by Friedländer in culture, but which we now know to have been the genuine lanceclate diplococcus, this very stain being one of the most valuable means of differentiating these two bacterial species from each other. The occurrence of both these organisms in the same case is not very uncommon. This was so in Friedländer's series and appears to have been true for the cases of acute lobar pneumonia reported by Weichselbaum as due to the capsulated bacillus. The capsulated bacillus grows much more rapidly and profusely than the Micrococcus lanceolatus, thereby inhibiting the growth of the latter. It is well known that it is often difficult to

cultivate the Micrococcus lanceolatus, especially from old pneumonias, and that it will grow only on certain media. Inoculation experiments are often negative, even with pure cultures of the micrococcus. We cannot, therefore, always depend on procuring in cultures the Micrococcus lanceolatus from the solidified lung, especially in older cases, even when it is present. In a large percentage of cases there is a general infection with the lanceolate coccus. We have cultivated it repeatedly from the heart's blood and various organs, even when it did not appear in cultures from the lung.

It is probably due to the careful observance of the following rules that we have found the Micrococcus lanceolatus so regularly in acute lobar pneumonia:

1. Several cultures on blood serum are taken from the solidified lung, both from the older and the fresher areas, also cultures from the heart's blood, kidneys, liver and spleen.

2. Coverslip preparations are made from various parts of the solidified lung, also from the pleural and, if present, the pericardial exudates, at least 3 or 4 preparations being made from each location, and stained for capsules, and in such Cases as No. 1 by the Gram stain.

3. Sections of lung and of other organs, hardened in Zenker's fluid and in alcohol, are stained for histological study, and for bacteriological study both by Gram-Weigert and with methylene-blue.

4. Inoculations of animals are made when the results of the bacteriological examination at the autopsy are not decisive as to the presence of the Micrococcus lanceolatus.

Unless similarly complete examinations are made the absence of the lanceolate coccus cannot be accurately determined in cases of pneumonia. The reports of those investigators who have found instances of acute croupous pneumonia, which they have attributed to the capsulated bacillus, cannot, in my opinion, be accepted, as in none of them, so far as I have been able to determine, have the foregoing requirements been rigidly carried out. We are, therefore, as the result of our investigations, of the opinion that all instances of genuine acute lobar pneumonia are caused by the Micrococcus lanceolatus.

This investigation was conducted under the direction of Professor Councilman, to whom I am indebted for many suggestions and much assistance. I am indebted for valuable aid also to Mr. W. J. McDonald, student at the Harvard Medical School.

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REPORT OF EXPERIMENTAL WORK ON THE DILUTION METHOD OF IMMUNIZATION FROM RABIES.

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As is well known, the dried-cord method of immunizing human beings from the active principle of rabies was first worked out and used by Pasteur. Since his death (and even for a time before) little progress has been made in elucidating the still obscure pathology of the disease or toward the discovery of the special germ causing it. The dried-cord method of immunization is the one used in Paris and in other places where the treatment has been introduced and applied to human beings.

In examining the literature of the subject I was much interested in the investigations of Högyes* in Budapest, by whom a dilution method was employed in a series of experiments for the production of immunization from rabies. His hypothesis is that, contrary to the teaching of Pasteur and others, the dried cord contains a dilution pure and simple and not merely an attenuated virus. Therefore, if this supposition be true, a fresh dilution made, under aseptic precautions, from the medulla of a rabbit dead from rabies, would be more exact, require less time, and be less liable to produce infection by accidental contamination from extraneous organisms which in the older method might develop during the drying process. In Pasteur's method the

* Högyes, *Acad. des Sciences de Buda-Pest*, Oct. 17, 1887. *Centralbl. f. Bakter.*, 1887, ii, 579. Abstract with critical remarks by Roux in *Annales de l'Institut Pasteur*, 1888, ii, 94.

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cords are kept from three to fourteen days in glass jars or bottles at a temperature of 68° to 72° F. in dry air, and then emulsified before use. Emulsifying a cord is a long, tedious process, and considerable exposure of the material is necessary.

The dilution method does not rest upon a sufficiently satisfactory experimental basis and has not been developed enough to have secured its general adoption in the treatment of supposed infection by rabies in human beings. This method as used in Budapest is carried out in the following manner: A piece of the medulla weighing one gramme is taken from a rabbit just dead with rabies. To this is added 10 grammes of sterile broth and then with a glass rod it is beaten into an emulsion. This is the stock solution from which the dilutions are made, and is of the strength of one part of fresh medulla to ten parts of broth. The dilution used first is 1-10,000, then on successive days 1-8000, 1-6000, 1-5000, 1-2000, 1-1000, 1-500, 1-250, 1-200, 1-100, and finally the full strength (1-10) is given. The quantity of each solution injected subcutaneously is usually the same. The dilutions 1-10,000 to 1-6000 are supposed to correspond in strength to a cord dried by the Pasteur method from 14 to 8 days. Subdurally injected, they produce rabies only exceptionally; the dilution 1-5000 inoculated subdurally does not kill all rabbits and those killed succumb only after a protracted period of incubation; the dilutions 1-2000, 1-1000, 1-500, 1-250, always kill and after gradually lessening periods of incubation. The dilutions 1-100 and 1-10 act as quickly as the very thick emulsion made from the medulla used to produce rabies.

The advantages claimed for this method over the dried-cord method of Pasteur are, chiefly, much greater accuracy in giving a gradually increasing dose, and, secondarily, less chance of infection after removal of the cord from the body, as the stock solutions are made fresh each day and the dilutions are made from them, whereas in the Pasteur dried-cord method the cords are exposed to atmospheric changes for from 3 to 14 days before being used.

The work in which I have been engaged consists in modifying the dilution method by keeping one stock solution, from which the dilu-

tions are made daily, throughout the whole course of treatment, instead of making a new stock solution each day as is done in Budapest. The advantages of this procedure over the Budapest method I believe to be the following: (1) Avoidance of the tedious process of making an emulsion daily; (2) ability to test before use the stock solution for extraneous organisms, which may produce serious symptoms; (3) and most important (if this method eventually proves successful), the possibility of sending out from one centre, where the material is prepared, the stock solution, with directions regarding dilutions and doses. The physician in charge can then treat cases which perhaps are not able to travel a long way from home for treatment.

TECHNIQUE EMPLOYED IN PREPARING THE EMULSIONS AND GIVING THE SUBDURAL INOCULATIONS.

As the result of considerable experimental work carried on during the last eight months (the tables of a part of which are given below), I now make and preserve a stock solution in the following way: The brain of a rabbit dead from laboratory rabies (fixed virus) is beaten into an emulsion composed of 8 parts of brain and 80 parts of sterile glycerine and water. The amount of glycerine used is $\frac{1}{5}$ part of the whole emulsion; at first $\frac{2}{3}$ glycerine was employed, then $\frac{1}{2}$, then $\frac{1}{3}$, and finally $\frac{1}{5}$, which last has been entirely satisfactory. As Table I (p. 185) shows, larger amounts of glycerine than $\frac{1}{5}$ part diminish the virulence of the virus, increase the incubation period, and prolong the lives of the animals inoculated. The brain, after being thoroughly beaten up and emulsified by the aid of a glass rod, is then poured into a sterile cheese-cloth bag and filtered, in this way making a smooth mixture with no visible particles. To this emulsion $\frac{1}{5}$ part of glycerine is then added and thoroughly mixed. The mixture is then placed in a sterile flask. During these manipulations every possible precaution is taken to avoid bacterial contamination. The flask is placed in an ice-chest. This, then, is the stock glycerine solution from which all dilutions are made. Before being used, the solution is tested for extraneous germs. Experiments have shown that a stock solution may be kept in this way seven weeks with no diminution in its virulence. As a course of immunization treatment lasts about 2 to 4

weeks, there can be no question of the virulence of the stock solution remaining in full strength for that length of time.

In nearly all subdural operations chloroform was used as an anæsthetic with perhaps 2 deaths in 50 animals. A collodion dressing has been found serviceable as a protection to the wound, and since using it no abscess of the brain has formed, as had previously occurred in a few cases. Instead of holding the animal during subdural inoculations by tying it down on a board by each leg, I have substituted an apparatus which has been used with satisfaction. The reason for the change was that tying a rabbit or a guinea-pig to a board causes the animal to struggle and become frightened and prolongs the operation. The holder which I have devised is a round tin cylinder 10 inches long and 6 inches in diameter. Into one end of this fits a block of wood hollowed out on the inner end to fit the hind part of the animal. This block is pushed into the cylinder, according to the size of the animal. At the other end, fitting around the cylinder, is a movable bag-like arrangement which is provided on the free end with a draw-string. On each side of the bag-like arrangement are straps which fasten to the block of wood by nails. These straps are provided with a number of holes to provide for the size of the animal. The draw-string which fits around the animal's neck, is pulled fairly tight, and the ends are fastened on two nails on each side of the cradle which holds the cylinder in place. In this way the animal is held securely in a telescope-like apparatus. A smaller size is preferable for guinea-pigs.

The virus used in the laboratory came from Dr. Ruhräh of Baltimore, and has been passed through a series of about 180 animals until now the rabbits show beginning paralysis on the 5th to 6th day; marked paralysis on the 8th day; complete paralysis on the 9th day, and death on the 10th day. It has become, in other words, a "fixed virus." In guinea-pigs inoculated with this fresh virus, rabies appears on the 6th day and death on the 7th day after inoculation. These periods of incubation are very constant when this virus is used.

The experiments detailed in Table I were undertaken to test the effect of different proportions of glycerine on the incubation period

of this virus kept for 1-7 weeks in a glycerine solution. At first $\frac{2}{3}$ part of sterile glycerine was added to an emulsion of the fresh virus, then $\frac{1}{2}$ part, then $\frac{1}{3}$, and finally $\frac{1}{5}$. Rabbits and guinea-pigs were the animals used for the experiments.

TABLE I.

EFFECTS OF DIFFERENT PROPORTIONS OF GLYCERINE ON DURATION OF INCUBATION PERIOD.

Proportion of Glycerine.	No. of Experiments.	Kind of animal used.	Date of preparation of virus.	Time solution was kept.	Effect on incubation period.
$\frac{2}{3}$ part.	3	Guinea-pigs.	July 7.	9 days.	Increased 2 days.
$\frac{1}{2}$ part.	3	Guinea-pigs.	July 15.	8 days.	Increased 1 day.
$\frac{1}{3}$ part.			Not sufficient to tabulate.		
$\frac{1}{5}$ part.	1	Guinea-pig.	Oct. 5.	7 days.	Not increased.
$\frac{1}{5}$ part.	1	Rabbit.	Oct. 5.	10 days.	Not increased.
$\frac{1}{5}$ part.	4	Rabbits.	Oct. 13.	7 weeks.	Not increased.
$\frac{1}{5}$ part.	2	Rabbits.	Oct. 25.	5 weeks.	Not increased.

Examination of Table I shows plainly that the proportion of $\frac{1}{5}$ sterile glycerine in the stock solution does not diminish the virulence of the solution during a period of seven weeks, rabies being produced at the end of this time with the same incubation period as that produced by an emulsion freshly made. This is an important point, because it shows that one stock solution may be kept through a whole course of treatment, which never lasts longer than 2 to 4 weeks. Many more experiments were made to test the virulence of solutions kept in this way, and made with dilutions from a stock solution; but the results simply confirmed those given and they need not therefore be repeated.

EXPERIMENTS IN IMMUNIZING ANIMALS FROM RABIES.

The principle of the dilution method is that inoculations of a very small amount of virulent material do not produce rabies, and that the gradual increase of the amounts injected accustom the animal to the most virulent material. As the stock solution is represented by one

part of medulla to 10 parts of sterile water and glycerine, we should in the immunization treatment take a small part of this and dilute it to 1-10,000 for the first treatment, giving part of a cubic centimetre subcutaneously to each animal as a first dose. For the second injection, the dilution is diminished to 1-9000, then to 1-8000, and so on, lessening the dilution each day. In Budapest the injections are begun with a dilution of 1-10,000 and the strength rapidly increased within the period of two weeks to a dilution of 1-10. In the first few series of experiments on immunization of animals I followed the scheme of dilutions and doses as described by the investigators in Budapest; but I now believe that the dilutions should be as follows: 1st day, 1-10,000; then upon successive days 1-9000, 1-8000, 1-7000, 1-6000, 1-5000, 1-4000, 1-3000, 1-2000, 1-1000, 1-900, 1-800, 1-700, 1-600, 1-500, 1-400, 1-300, 1-200, and then this last dilution employed until treatment has been continued 2 to 4 weeks. Any dilution below 1-200 is likely to produce rabies. The dose of these various dilutions should be proportioned to the size of the animal. It should be noted that guinea-pigs are very susceptible to the virus of rabies, apparently much more so than rabbits. After finishing the immunization treatment in each series of animals, a subdural inoculation of fresh virus has been made in nearly all the cases, as a test of immunity. These inoculations have been made in some animals immediately at the end of immunization and in others within 2 weeks of its cessation. A guinea-pig or rabbit subdurally inoculated with fresh laboratory virus invariably dies, unless previously immunized, as has been proven in a large number of experiments. If, therefore, after a subdural inoculation with fresh laboratory virus the animal does not die within 10 days, we may feel sure that immunity has been secured by the treatment given. The subdural inoculation test is the most severe we can employ for testing the immunity of an animal.

Table II shows the results in different series of animals treated by the glycerine dilution method to produce immunity from rabies. In the first experiment, two to three stock solutions were used during the course of the treatment and dilutions made from them. In the last two series, one stock solution has been kept throughout and the

daily dilutions made from it, beginning with a dilution of 1-10,000, and then gradually increasing the strength as described above until 1-200 was reached in the case of the guinea-pigs and 1-100 in the case of rabbits.

TABLE II.

RESULTS OF IMMUNIZING TREATMENT BY DILUTION METHOD.

Number of animals in series.	Duration of treatment.	Highest and lowest dilutions used.	Animals died rabid during treatment.	Died from other causes.	Immune from effects of subdural inoculations after treatment.
A. 4 guinea-pigs	14 days	1-10,000 1-10	None	None.	None.
B. 6 rabbits	4 weeks	1-10,000 1-10	None	5 *	1
C. 9 guinea-pigs	3 weeks	1-10,000 1-10	5	1	1 †
D. 40 guinea-pigs	20, 3 weeks 20, 4 weeks	1-6,000 1-10	20	2 1 lost	17 ‡
E. 6 rabbits	3, 3 weeks 1, 4 weeks	1-10,000 1-100	None	1 injured 1 lost	1
F. 50 guinea-pigs	20, 3½ weeks 30, 4 weeks	1-10,000 1-200	None	3 suddenly 2 injured	20 §

In the final inoculations to test immunity control animals were inoculated in the same way as the treated animals, and all died from rabies in 7 to 10 days. It will readily be seen from Table II that, while immunity can be produced by this method of treatment, in several instances rabies was produced by the treatment. This result, in my opinion, annuls any advantages this method may otherwise have over the dried-cord method of Pasteur.

The foregoing results were obtained during the year 1897 and the investigations were continued during 1898, with both the dilution and the dried-cord methods. I have had good opportunity to test the dried-cord method of Pasteur during the past eleven months.

The Health Department of New York City has adopted the Pas-

* This series was treated during the heat of the summer when unused animals in the laboratory died in large numbers from no apparent cause. Inoculations made from these animals showed no evidence of rabies.

† The other two animals in this series received subcutaneous inoculations with fresh virus, and lived.

‡ All the animals which lived through the treatment were immune from effects of subdural inoculations.

§ 25 animals were not immune from effects of subdural inoculation.

teur antirabic treatment and during the past eleven months I have treated thirteen cases by the Pasteur method, the first six in conjunction with Dr. Robert J. Wilson. Besides the treatment of human beings experiments upon animals have been conducted in order to test the Pasteur method and various modifications of it. The results have been in general confirmatory of those reported from the Pasteur Institute in Paris. In order to make sure of the absence of contamination from the inoculated material we have not only tested the cords before using, but have also tested the emulsion of the cord after it had been prepared and have awaited for 18 to 24 hours the results of the bacteriological test, the emulsions during this period being kept on ice, a procedure which can be followed without any impairment of the virulence of the emulsion, as we have proven experimentally. Besides these precautions, animals can be simultaneously treated with the same material as that given to the persons under treatment. Under these precautions we have observed no local or general disturbance whatever after the injections. In four instances the emulsions of the dried cords made each day were sent by a special messenger out of town packed in ice and were used with perfectly satisfactory results. We have thus secured by the dried-cord method the special advantages claimed for the dilution method without the dangers of the latter.

CONCLUSIONS.

1. I have simplified the dilution method by using a stock glycerine emulsion of the virulent cord, from which the desired dilutions can be readily prepared. The proportion of glycerine should not exceed $\frac{1}{5}$ part, if it is desired to retain the full virulence of the emulsion.

2. There is some danger of giving rabies to animals in the dilution immunization treatment, a danger which is not present in the Pasteur method.

3. The dried-cord method does not rest solely upon the principle of dilution, but is based also upon attenuation of the virus.

4. The Pasteur method being entirely free from the element of danger which pertains to the glycerine dilution method and resting upon a sounder experimental basis is the one to be preferred.

A STUDY OF THE SPINAL CORD BY NISSL'S METHOD IN TYPHOID FEVER AND IN EXPERIMENTAL INFECTION WITH THE TYPHOID BACILLUS.*

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Plates II–IV.

This paper presents the results of the study of certain changes noted by means of Nissl's staining method in three cases of typhoid fever and in a series of experimental inoculations of rabbits with Bacillus typhosus. My observations relate mainly to the alterations shown by this method in the motor cells of the ventral horns of the spinal cord and in the nerve cells of the dorsal root ganglia. With the Nissl method only one constituent of the cell body is stained by the basic dye, and hence this is called the stainable or chromatic substance. In the normal nerve cell of the motor type this consists of coarse, spindle-shaped masses which are regularly distributed throughout the body of the cell, and, with their long axes more or less parallel, run from process to process. On closer examination these masses, called the "Nissl bodies," are seen to be composed of an aggregation of small deeply staining granules (Held, 14). Into the dendrites these masses are continued less thickly distributed and drawn out into thin threads, plastered against the wall or angle of some branch, or coursing through the middle of the process. The axone on the other hand is entirely clear of stained particles, as is also a small area about its origin, known as the "axone hillock." The nucleus occupies the centre of the cell and, with the exception of the nucleolus, contains normally no substance stainable by Nissl's method. The nucleolus is small, sharply outlined and very intensely stained.

The points to be emphasized, as regards the normal cell, as seen by

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this method, are the fairly constant size of cell body, nucleus and nucleolus, the even, regular distribution and distinct character of the Nissl bodies, the central position of the nucleus, the clear nuclear substance and the small deeply staining nucleolus.

The stainable constituent of the cell body is now generally believed to represent a nutritive substance, previously soluble in the fluid portion of the cell, but precipitated by fixing agents as incrustations upon the achromatic or unstained portion. The achromatic portion of the body of the cell is regarded by Marinesco (22, etc.) as an unstained network prolonged into the processes as minute fibrils, which represent the conducting portion of the cell. Van Gehuchten (9) would account for the regular shape and arrangement of the chromatic bodies by supposing them to be incrustations, especially at the points of intersection of this network.

It obviously makes no difference in the results of our study of pathological effects whether we believe these structures to exist definitely as such in the cell during life, or to appear only after death as the result of fixing agents. What is of importance is that they possess in the normal cell a definite arrangement, constant for a variety of fixing agents and stains, though best shown by the Nissl method (Flatau, 6).

The normal cell shows variation from this type chiefly in the amount of chromatic substance. When this is present in larger amounts, the condition has been termed by Nissl (29, 30) "pyknomorphism," and has been thought to represent a state of rest. When, on the other hand, the nutritive element has been used up and chromatic substance is scanty, he has termed the condition "apyknomorphism." This variation in amount of chromatic substance does not alter the regular shape or regular distribution of the Nissl bodies. When these are found broken apart and destroyed, the condition is pathological, and for it Marinesco (21) has suggested the term "chromatolysis."

METHODS.

In the cases of typhoid fever to be described sections were taken from the cervical, thoracic, and lumbar regions of the cord and their respective dorsal root ganglia when practicable. Small pieces of the tissue

were placed at once in 95 per cent. alcohol and allowed to remain there from 12 to 20 hours. They were then transferred to absolute alcohol, and thence to a mixture of equal parts of absolute alcohol and xylol, remaining in each 12 to 20 hours, the longer period being more desirable, as it produces less distortion. After 5 hours in xylol they were kept in melted paraffin at about 55° C. for from 3 to 4 hours, and then embedded in paraffin having a melting point of 52° C.

The sections were stained in Nissl's methylene-blue solution, and differentiated in 0.1 per cent. alum solution, as recommended by Held (14). For mounting benzine colophonium was used, the benzine being driven off with as little heat as possible.

THE HUMAN CASES. SPINAL CORD.

Of the three human cases the first was of ordinary character, but a very severe infection; the second a severe infection ending in perforation and sudden death; the third a very severe infection in an old man, unrecognized before autopsy. The experimental material was derived exclusively from rabbits.

Case I. Man, aged 28 years, who died in the hospital after a severe attack lasting 11 days from date of onset. The ordinary signs of typhoid fever were present, and there were no complications except unusual nervousness and delirium, and a short time before death, difficulty in swallowing, which may be accounted for by ulcers in the œsophagus found at autopsy.

The autopsy was made 6 hours after death, the body having been preserved on ice. In addition to intestinal ulceration, ulcers were found in the gall bladder, stomach, œsophagus, larynx, and on the tongue.

The bacteriological examination showed pure cultures of Bacillus typhosus in the liver, spleen, mesenteric and retroperitoneal lymph glands, and urinary and gall bladders. The ulcers of the œsophagus and larynx contained streptococci and staphylococci.

The number of altered nerve cells in the cord is very numerous, but in most of these the alterations are not marked, except in the lumbar region of the cord. The size of both cell and dendrites is increased; in the more advanced stages the cell body is considerably enlarged. In the cell there has been a breaking apart and partial or complete solution of the chromatic substance or the Nissl bodies,

advancing from the region of the nucleus out toward the periphery. The component granules of these bodies not merely are separated and partly destroyed but also appear more or less diffused in the fluids of the cell, so as to form when precipitated pale, blurred flocculent-looking masses. [Plate II, Fig. 1.]

This process has not gone on to the same extent in all cells. Some, notably in the cervical and thoracic regions, vary hardly at all from the normal type. Others, and these in great numbers in the lumbar region, show an advanced stage of this form of chromatolysis. In this region wherever the axone with its hillock is accurately determined, the dissolution of the granules is especially marked in that portion of the cell, and, wherever pronounced, the nucleus has migrated somewhat toward the opposite side of the cell. In these cells the slightly enlarged dendrites are almost free from chromatic substance, rendering the determination of the axone, although not a more difficult problem, yet one requiring more care. [Plate II, Figs. 1 and 2.]

The achromatic substance has been altered in some parts but very slightly, and in such a way as to stain very faintly with the methylene-blue, a condition which, with the absence of the chromatic substance, serves to show more clearly its structure. In the dendrites and body of the cell, this staining is of a more indefinite nature; about the axone hillock, the staining is granular or net-like; in the axone, in the form of very few and fine, parallel, granular streaks. (Marinesco, 22.)

The nucleus is not enlarged in the cells of less advanced chromatolysis. Under the low power in the nuclei of most of these cells there is apparently a diffuse blue stain. This, under the high power, resolves itself into minute, faintly staining granules. In the more advanced stages the nucleus, in addition to being often eccentric, has become considerably enlarged, while the granular deposit is not increased and therefore is less thickly and more unevenly distributed within it. In all stages the nucleolus is greatly enlarged and less deeply stained than normal. In many of the cells it is so faintly stained and so vacuolated as to be scarcely visible, while in others it is undergoing complete disintegration.

Case II. Woman brought to the hospital suffering from typical typhoid fever. The disease ran an ordinary course until the morning of the 10th day, when the patient sank rapidly with profuse sweating, vomiting, dull delirium, distended and tender abdomen and died at 7.30 that night. There was a leucocytosis of 22,600.

At autopsy, 14 hours after death, a perforation of the ileum, with a consequent general, sero-fibrinous peritonitis, was noted in addition to the usual post-mortem appearances of typhoid fever.

Typhoid bacilli were cultivated from the bile, spleen, mesenteric glands and kidney; Bacillus coli from the lung, peritoneum, kidney, spleen, and liver.

Obviously the secondary infection of the peritoneum makes this not an absolutely pure case, but in view of the long period of infection with the typhoid organism and the shortness of the secondary infection, the latter can be practically disregarded.

The changes in the ventral horn cells are so nearly like those of Case I that they may be dismissed with a few words. In the numbers of the cells affected and the characters of the degeneration the two cases are nearly identical. The only difference is in a slighter degree of alteration in the nucleoli, these retaining in Case II a deeper stain and more definite outline. In some cells, although the chromatolysis spreads inward from the axone hillock, there are a few large abnormal blocks of chromatic substance filling in the corners formed by the entrance of the axone and the periphery of the cell.

In the two foregoing cases, in spite of the great and early disappearance of chromatic substance from the dendrites, by far the greater number of cells show the central form of chromatolysis. The point of entrance of the dendrites is in nearly all cases blocked by the remains of Nissl bodies, which had been altered much less than those in the more central portion of the cell [Plate II, Fig. 1]. There are a few cells, however, which show a more complete destruction advancing from the periphery and in which the nuclei are not eccentric. It was noted, however, that this appearance is, in some cases, in sections which do not show the nucleus, but have passed through the dendrites at the periphery of the cell, and that, in the succeeding section or sections, as one approaches the nucleus, this appearance gives place to the central form of chromatolysis. In cells showing this peripheral

character of chromatolysis the nucleus when present in the section is not eccentric, but it seems to be undergoing disintegration in the centre of the cell.

Case III. Man, aged 67 years, who entered the hospital in a dull, listless state, soon passing to unconsciousness, and from whom no satisfactory replies could be obtained. Friends stated that he had been ailing for two months with loss of appetite and weight. For two weeks before admission he had pains in the back, indigestion and shortness of breath and was very thirsty. Physical examination showed a large area of dulness in the right lower lobe with friction and tubular breathing. There was no expectoration. There was a leucocytosis of 15,000. The pulse was 128, while the temperature rose toward evening to 104° F. These facts naturally led to the diagnosis of senile pneumonia. The patient died two days after admission, and the autopsy was made within six hours after death.

At autopsy there was found a recent thrombus in the branch of the pulmonary artery supplying the lower lobe of the right lung, and consolidation of this lobe with gangrene and perforation of the visceral pleura. The spleen was enlarged and soft, but no intestinal lesions were present. The bacteriological examination, however, revealed the presence of Bacillus typhosus in the consolidated area in the lung, in the spleen, and in other organs. This case, which will be reported in detail elsewhere, is of especial interest as an example of infection with the typhoid bacillus without intestinal lesions.

Unfortunately the cord in this case could not be removed in its entirety, and therefore only as much as could be taken out through the cranial cavity could be reserved for examination.

In this case the earlier alterations described in the first two cases can be traced in only a few cells. The vast majority of cells show a much more advanced stage of alteration. These cells are swollen and distorted in varying degrees. The central portion of the cell is a mass of ill-defined, extremely pale, flocculent-looking material, studded everywhere with small round refractive bodies, probably representing remains of the normal achromatic network [Plate II, Figs. 3, 4 and 5]. Scattered along the periphery are a few small and large clumps of pale washed-out looking granules undergoing the later stages of disintegration and solution.

The nucleus is large and swollen, and sprinkled with the finely granular deposit. It no longer occupies a position anywhere near the centre of the cell, but bulges from the periphery. Along its internal margin there is often a fine dark line composed of minute deeply staining granules [Plate II, Figs. 3 and 4]. The nucleolus is pale and vacuolated and frequently may be seen to protrude from the prominent nucleus. In some few cells there seems to have been an actual extrusion, the nucleolus lying free with the remains of the crumpled nuclear membrane about it [Plate II, Fig. 4]. This latter appearance, however, may be due to tearing with the knife, although no injury to the surrounding tissue can be made out.

There seems to be a marked disappearance of many processes from the cells, perhaps an illusion due to the great increase in size.

THE DORSAL ROOT GANGLIA IN HUMAN CASES.

The consideration of the dorsal root ganglia in these cases, as elsewhere, is attended with certain difficulties. These are, chiefly, the absence of processes, and the great variation under normal conditions in the size of the cells and in the amount and distribution of the chromatic substance. Thus Lugaro has divided these cells into five normal types. For this reason it has been thought better to notice only such alterations as are well marked, and to discard all those of a doubtful nature. Owing to the refusal of permission to remove the cord in the third case the ganglia could not be studied. The following statements, therefore, are confined to Cases I and II, that is, to the ones showing less alteration in the cells of the ventral horns.

The alterations of a definite nature are identical in both cases and present in only a few cells. They consist mostly in a rarefaction of the chromatic substance and extremely eccentric position and even bulging of the nucleus. A mass of chromatic substance is often plastered against the cellular margin of the nucleus, which, though large, is much shrivelled, often full of a granular deposit, and contains a very large pale vacuolated nucleolus [Plate III, Fig. 12]. In addition a small number of cells show absence of chromatic substance in a central zone between a ring of large globular-looking bodies about the

periphery, and a collection of similar bodies immediately about the nucleus (as in Rabbit VI, Plate IV, Fig. 16).

EXPERIMENTAL CASES. SPINAL CORD.

In order to determine the constancy and mode of progress of the previously described lesions in typhoid infections the following inoculatory experiments were made on rabbits:

Inoculations were made into the ear vein, using bouillon cultures of the typhoid bacillus derived from the second human case. The following table shows the character of each experiment:

TABLE

SHOWING HISTORY OF INOCULATED RABBITS.

Number of Animal.	Dose.	Remarks.
I.	2 cc. 80-hr. culture.	Very intense reaction. Died in **2 hours.**
II.	2 cc. 70-hr. culture.	Ordinary reaction. Killed in **7 hours.**
III.	2 cc. 96-hr. culture.	Ordinary reaction. Killed in **33 hours.**
IV.	1st day, 2 cc. 2-day culture. 3d day, 2 cc. 5-day culture.	Ordinary reaction after first dose. After second dose animal rapidly succumbed in a few hours, *i. e.* **52 hours** after first inoculation.
V.	1st day, 2 cc. 2-day culture. 3d day, 2 cc. 5-day culture.	Ordinary reaction after first dose. After second dose animal extremely weak; lost weight for several days. At end of first week seemed very feeble in hind legs. As symptoms of convalescence began to appear, animal was killed **9 days** after first inoculation.
VI.	First 5 days, 1 cc. 6th and 7th days, 2 cc. 8th day, 15 cc. into peritoneum.	Animal very resistant. Blood agglutinated rapidly at end of 7th day. On 8th day 15 cc. were injected into peritoneal cavity without effect. On 23d day paralysis of hind legs first noted. Animal killed and autopsy made **24th day** after first inoculation.
VII.	2 cc. 72-hr. culture.	Animal passed through first effects and was allowed to live. About 29th day paraplegia of hind legs first noted; great emaciation of rump and legs. As symptoms of recovery set in, animal was killed on **64th day** after first inoculation.

As will be seen by consulting the table an attempt was made to get the degrees of toxic effect at different periods.

In *Rabbit I*, 2 hours after inoculation, the changes though noticeable are slight and confined to a few cells. They consist of slight swelling of the Nissl bodies and a tendency to disintegration and disturbance of their orderly arrangement.

In *Rabbit II* these alterations, already in 7 hours, have become marked, and notably so in the lumbar region of the cord. They correspond in nature, if not in degree, to those already described in the first and second human cases, and consist, as before, in the pale washed-out form of chromatolysis starting from the axone hillock and displacing the nucleus slightly to the opposite side [Plate III, Fig. 7]. The nucleus shows slight enlargement and the finely granular deposit. The nucleolus is large, pale and vacuolated. The enlargement of the cell and dendrites, the disappearance of chromatic substance from the latter, and the alterations in the achromatic substance are not pronounced.

In *Rabbit III*, which had been subjected to infection for the still longer period of 33 hours, the number of normal cells is greatly reduced, and it becomes evident at once that we have to deal with two different kinds of alteration. Most of the altered cells present the same appearance of central disintegration and solution of the granules spreading from the axone hillock, with the accompanying changes in nucleus and nucleolus, already described in the human cases and the second animal.

An appreciable number of cells, however, present the type of peripheral destruction observed in a few cells of the first two human cases. In some of these the cell body and processes are somewhat contracted and their outline is no longer regular. In the periphery of the cell any distinction between chromatic and achromatic portion is almost lost, the whole substance appearing granular and shred-like, with here and there small chains of disintegrated granules [Plate II, Fig. 6]. Toward the centre of the cell these chromatic masses become more and more abundant, until about the remains of the nucleus they are quite densely packed. The appearance in some of these cells is as if, progressing from the periphery, the Nissl bodies had been broken apart and destroyed *in situ*, not washed out into the surround-

ing substance. The nuclear membrane has disappeared, and the only sign of the position of the nucleus is a slightly rarefied area containing a dark, disintegrating nucleolus.

In some of these cells single or multiple clear areas resembling vacuoles may be observed [Plate II, Fig. 6]. These correspond, presumably, to those described by many observers in a variety of experimental intoxications, and seen by Babes (1) in typhoid and in other experimental infections to contain the organism used for inoculation. If present in large numbers such appearances may be significant. In my study this appearance was observed in one cell only, and in this animal, and is depicted in Plate II, Fig. 6. Other cells of this type show very little irregularity of outline or contraction, while the achromatic substance does not seem much altered. The granules are paler and more washed-out looking, though more numerous and in larger groups about the nucleus, which also is not much altered.

The whole gray matter of the cord of this rabbit is infiltrated with leucocytes, mainly small mononuclears, which are very abundant in the small dilated capillaries, in which they may be seen in the act of migration, and thus to invade the pericellular lymph spaces.

In *Rabbit IV*, which succumbed after a period of 52 hours, nearly all the cells are affected; practically no normal cells can be found. The type of chromatolysis which begins at the periphery, however, has almost disappeared, while the other or central form has become very prominent. The former lesion, that characterized by peripheral destruction, in addition to affecting fewer cells than in Rabbit III, differs also in the absence of any greatly contracted cells and of the appearance of vacuolization described in the foregoing animal. The cells resemble the other variety of this type. They are perhaps slightly enlarged, pale, diffusely blue, with a few flakes of chromatic substance grouped about the nucleus, which is little altered.

The latter type of lesion, that characterized by central chromatolysis, in addition to affecting a greater number of cells than before, has advanced considerably in the individual cells. These are large and swollen as well as their dendrites, which are nearly free from chromatic substance. At times one of two dendrites, which join to enter

the same cell, is almost entirely deprived of chromatic substance, while the other contains more or less well-formed Nissl bodies, a difference which exists even after their juncture, and before the cell body proper is reached. The disintegration and washing out of the granules has progressed pretty thoroughly throughout the cell, leaving but a faintly marked zone of slightly larger collections of granular debris at the periphery. Where the axone is cut this alteration as before may be seen to extend outward from the axone hillock.

The nuclei are only slightly eccentric, and show the same general appearance of a deposit, grouped especially about an irregularly swollen nucleolus [Plate III, Fig. 8].

Rabbit V lived for 9 days after the first inoculation and during this time lost much weight and nearly all control of the hind legs. The animal was killed as symptoms of recovery began to appear. The ventral horn cells in the lumbar region showed, contrary to expectation, very few cells similar to those already described, although great care was taken to cut in sections all parts of this region. Nevertheless, in that portion just above the entrance of the sciatic nerve, groups of large swollen cells were found with slightly eccentric nuclei, but in which Nissl bodies of characteristic shape and distribution were arranged somewhat concentrically about the nucleus, with a slight intensification of single granules in that region. These granules are bright and freshly staining, and their arrangement is very regular [Plate III, Fig. 10]. From their position in the cord these cells probably send fibres into the sciatic nerve. Alterations found in this nerve and the large size of these cells seem to indicate that they must have undergone previously some change and probably that of the central type already described [Plate III, Fig. 8]. The newly formed, regular appearance of the Nissl bodies, taken in consideration with the fact that this animal was recovering, suggests strongly the idea that these cells are undergoing a process of restitution. There is no infiltration with round cells demonstrable.

Rabbit VI proved very resistant to repeated inoculations and was allowed to live. There developed gradually paralysis of both hind legs, which was first noted on the 23d day. The emaciation of the

muscles of the rump and legs was very marked. On the 24th day the animal was killed and autopsied. The alterations found in this case are of interest both on account of their character and their distribution.

In the cervical cord there are scarcely any pathological changes. The cells, however, are in an apyknomorphic state, that is, the chromatic material seems to have been used up to a considerable extent, but the normal arrangement still remains. There are, moreover, some cells which are larger than others and present a slight suggestion of chromatolysis about the axone hillock and in the dendrites.

In the thoracic portion of the cord there is nothing of note.

In the lumbar enlargement most of the cells are of the normal type, and, in contrast to those of the cervical portion, are in an extreme state of pyknomorphism. The Nissl bodies are very numerous, in many cells slightly smaller than usual, and connected by small threads of granules forming a dense network of chromatic substance. A very few cells are much enlarged, and the chromatic substance is disintegrated and undergoing dissolution pretty evenly throughout the cell. The nuclei and nucleoli in these, as well as in some of the cells of more normal type, present the same general changes as those described in the previous cases.

But in the immediately adjacent portion of the lumbar enlargement, just above the entrance of the sciatic nerve, there is a localization of marked alterations, corresponding, it may be assumed, to the area of nervous control of the paralyzed muscles. A number of cells are still normal or only slightly changed, but here and there mere shadows of degenerated cells may be seen, with a number of small lymphoid cells apparently about to invade their territory.

In addition to these two extremes, the one of health, the other of extreme degeneration, altered cells are numerous, and indeed in a very advanced stage of pathological change. The cell and processes are enormously swollen, and the latter, many of which have apparently disappeared, are nearly completely free from chromatic substance. In the central portion of the cell the chromatic substance has been almost completely washed out, leaving a few flakes and pale, minute granules

scattered about in a faint, diffusely blue background. The periphery is bordered by a zone where these flocculent, pale granules are slightly more numerous [Plate III, Fig. 9]. The nucleus in the majority of these cells is partly protruding from the periphery, and encloses a very irregular, disintegrating nucleolus, surrounded by the finely granular blue deposit already mentioned. Out-side the nucleus and along its cellular margin there is generally a fine layer of more deeply staining granules [Plate III, Fig. 9].

In certain of these cells, notably in those where the nucleus is not bulging from the periphery nor very eccentric, the fine deposit within the nucleus assumes a metachromatic, pale violet stain. By the concentration of these fine violet granules along the internal surface of the nuclear membrane, the nucleus is thrown into sharp relief against the pale blue of the more central portion of the cell. Some of these nuclei are large and only slightly irregular in outline, others small and greatly shrunken and distorted. As in the others the nucleoli are pale and undergoing the last stages of disintegration. In some of these latter cells, three zones of chromatolysis may be sharply distinguished, a very clear one immediately about the nucleus, a second not quite so clear separating it from the third or the border zone of larger masses at the periphery already referred to.

Rabbit VII, the last of this series, was first noticed to be paralyzed on the 29th day. Symptoms of recovery becoming marked, the animal was killed 64 days after the first inoculation.

In the cervical cord the ventral horn-cells do not differ in great degree from the normal type, although a number of slight variations exist. These consist for the most part in a slight increase in size of the cell, and a little irregularity in the shape and distribution of the Nissl bodies. This irregularity seems to consist, not in a breaking apart and destruction of the granules, but rather in the addition of new sharply staining granules to those already present, causing a little interference with the regular striped appearance of these bodies. This is usually accentuated about the nucleus, which is a little swollen and contains a few bluish granules, but is otherwise normal. Some cells are very deficient in the chromatic substance, especially about the

periphery. The chromatic granules about the nucleus in these cells are distinct and stain brilliantly with the methylene-blue.

At about the level of the second lumbar vertebra the cells are for the most part well formed and appear vigorous. The chromatic substance is dense and abundant, and characteristically arranged. The nuclei of these cells are of normal size and assume a very finely granular, almost homogeneous stain. Here and there are a few smaller cells with the chromatic substance distinct but somewhat finely divided.

On reaching approximately the level of the third lumbar vertebra a very different and striking picture is obtained. Corresponding doubtless to the paralyzed area are found large groups of cells which have a strong resemblance in their great size, eccentric nuclei and large clear dendrites to the cells in about this region in the previous experiment [Plate III, Fig. 9]. Here, however, the resemblance ceases. Instead of the pale, glassy, washed-out centre, the distorted, shrunken or swollen nucleus, and disintegrating nucleolus, the cell is filled with brightly staining sharp chromatic granules arranged in streaks shaped like the Nissl bodies, and traversing in a parallel direction the body of the cell toward the dendrites, or concentrically disposed about the nucleus, where the chromatic substance is especially abundant, being often especially dense along the inner edge of the nucleus [Plate III, Fig. 11]. The nucleus, instead of being greatly altered, is large, regularly spherical or oval, contains only a slight granular deposit and a normal deeply staining nucleolus. Many gradations in the amount of chromatic substance may be seen, but all these cells show this regular, apparently new formation of chromatic substance.

That these cells, as well as some of those described in the cervical region, are in a state of regeneration or restitution, is highly probable from their appearance alone, which corresponds also with that described as such by van Gehuchten (9), Marinesco (21) and others. In connection with this should also be taken into consideration the fact that this animal showed marked improvement, although the clinical symptoms and the alterations in the Nissl bodies are not always correlated.

A few cells seem to have lost their nuclei and to have completely degenerated. These are pale, shrunken forms contained in clear spaces, with a few blue flakes scattered within them, and at times invaded by wandering cells.

The part played by the nucleus in the stages of degeneration and of restitution is interesting. As is well known, if the nucleus be intact the cell may retain its power of regeneration. Should the nucleus, however, become completely degenerated or extruded from the cell, this power is lost (Nissl, 28). In many of the most altered cells, the nuclei which show marked signs of degeneration, as for example the metachromatic alteration in the granular deposit, are surrounded by a zone entirely free from chromatic substance, while in regenerating cells about the normal-looking nucleus, new granules are especially abundant [Plate III, Fig. 11]. In other words, as the nucleus gives out, the granules disappear from about it; as the nucleus recuperates, a new crop of granules appears along its cellular margin. This looks as if the nucleus controlled their formation, and thus, upon the supposition that they represent a nutritional element, presided in some way over the nutrition of the cell. The appearance of granules within the nucleus of altered cells may have something to do with this function, and may represent an exertion to replace the excessive destruction of the chromatic substance.

THE DORSAL ROOT GANGLIA IN EXPERIMENTAL CASES.

The dorsal root ganglia showed changes of a marked nature in the last four of these animals. In the ganglia from the lumbar region the altered cells are numerous. They are chiefly of two kinds and correspond in part to those already described in the human cases.

A few cells show a clumping of the chromatic granules into large swollen masses arranged in a row around the periphery and gathered about the centrally placed and only slightly altered nucleus. Between these two situations is a zone entirely free from chromatic substance [Plate IV, Fig. 16]. Others show no peripheral zone, but the chromatic substance is massed about the nucleus in the centre of the cell [Plate IV, Fig. 17].

The majority of the altered cells, however, show eccentric nuclei. In some few of these the chromatic substance is scattered at intervals in large flakes through the body of the cell [Plate IV, Fig. 15]; in others, the chromatic substance is finely divided, and in some of the latter the destruction seems to begin in one or more small areas [Plate IV, Fig. 13]. The nucleus is very eccentric, frequently bulging from the periphery, and encloses a pale, very irregular nucleolus. Altered cells, showing in particular eccentric nuclei, are most abundant in Rabbit IV, where most of the cells of the ganglia are thus affected.

In Rabbit V, on the contrary, there are few alterations, but there is great uniformity in the appearance of the cells, which are for the most part rich in chromatic substance, especially about their nuclei. This difference in the ganglia in these two animals corresponds very closely with the difference in the same animals between the conditions of the ventral horn cells.

In Rabbit IV, which died under the influence of an acute attack, the chromatic substance in both situations is much reduced; Rabbit V was on the road to recovery and an apparent restitution of chromatic substance is observed. This correspondence of external manifestations and internal alterations is not always so exact. The Nissl bodies are in a state of extremely unstable equilibrium and very easily influenced quantitatively. When such correspondence does exist, however, it is worthy of consideration.

The similarity of these alterations in the inoculated animals with those found in the human cases needs no further comment. The lesions are evidently uniform and constant. In both the human and the experimental series, the ventral horn cells show two forms of lesion. One is indefinite and characterized mainly by contraction or slight, irregular enlargement of the cells; by a chromatolysis marked in the periphery of the cell and at the entrance of the processes, with disintegrated granules more numerous about the nucleus which is either normal or together with the nucleolus is undergoing destruction in the centre of the cell. In some of these cells a process of vacuo-

lization is noted, and an apparent degeneration of the achromatic portion.

The other type of lesion is definite and well characterized by a regular enlargement of both cells and processes, a pale washed out chromatolysis starting from the axone hillock and surrounding the somewhat altered nucleus, which is displaced to, and often protruding from, the opposite side. The achromatic portion is but little altered.

The first type of lesion is at no time predominant. It is most marked, however, in Rabbit III, and decreases somewhat in Rabbit IV. Both these animals were killed when the influence of the toxic substance on the whole system was at its height.

Without entering into a full discussion of the direct effect of toxic substances upon the nerve cell, attention may be called to the general resemblance between the alterations of this first type and those which have been produced by a number of investigators in different experimental intoxications, such as by arsenic, strychnine, alcohol, etc. These changes are doubtless due to the direct action of poisons on the cell. For a discussion of this subject reference may be made to the excellent articles of Marinesco (21), Lugaro (16), Flatau (6), and Goldscheider and Flatau (12). Although slight differences exist in the descriptions given by different authors, yet the lesions are essentially of the same type, and no doubt in our typhoid cases, too, they may be attributed to the direct action of the typhoid toxine.

Babes (1) has studied the changes in one case of typhoid inoculation in connection with a study of other infections, and describes only this first form of lesion, which he believes to be due to an actual invasion of the cell, as well as the tissues of the cord, by typhoid bacilli. Probably to this class belong especially the irregularly contracted and vacuolated cell forms with disintegrating nuclei.

Goldscheider and Flatau (10) and Moxter (25) have produced by experimental elevation of temperature, and Goldscheider with Flatau (11) and with Brasch (13), Déjerine (4), and Marinesco (20) have found in certain affections with high temperature in man, an alteration which corresponds closely with the pale blue non-vacuolated variety here described. Perhaps these forms may be referred to this

effect, though the temperature of the animals, when taken, was never particularly high.

Whether this lesion is due to direct toxic action, to high temperature, or to some other condition or to a combination of these need not be further discussed here. It is the more indefinite and not the predominant and characteristic lesion.

The second and characteristic lesion is that which predominates in all the human cases, and is especially advanced in the last of these. It is present to a greater or less degree in the whole animal series. It first shows itself in Rabbits I and II, to increase in III, to become particularly abundant in IV, when the other lesion begins to disappear. Its traces may be seen in the enlarged, apparently restituted cells described in Rabbit V, in which recovery from a half paralyzed condition was taking place. It is particularly advanced, though confined to the lumbar region, in Rabbit VI, which developed a paralysis of both hind legs. In Rabbit VII, paralyzed but recovering, it was manifested in the large cells, with eccentric nuclei, which were apparently undergoing regeneration.

This lesion, in its mode of onset, its general characteristics and progression, is similar in every way to the alterations produced in the corresponding cells in consequence of experimental section or other interference with the function of the axone, as described by Nissl (26, 27), Onufrowicz (31), Marinesco (21), Dutil (2), and many others. Specimens made in this way by Mr. Erlanger of the Johns Hopkins Medical School were kindly lent to me for purposes of comparison.

Moreover, an identical lesion has been discovered in human beings, where the axone has been injured or its function disturbed. Thus, Flatau (7) found this condition in two cases of amputation, and in one of thrombosis of the femoral artery; Adolf Meyer (24) in the facial nucleus, where the nerve had been compressed by inflammation in the internal auditory canal; and Barker (3) after injury to the fibres of the direct cerebellar tract in spinal meningitis found the same change in the cells of Clark's column.

Alterations in the dorsal root ganglia have also been studied after section of their peripheral nerves. Although this branch does not

correspond to the axone of the motor cell, yet it is apparently the only one which, when severed, produces alterations in the appropriate ganglion, as Lugaro (15) has shown by section of the cord above the ganglion examined, and by section of its peripheral nerve. By the latter procedure Lugaro observed alterations similar to those of cells with eccentric nuclei described in our typhoid cases, and shown in Plate III, Fig. 12, and Plate IV, Figs. 13, 14 and 15. R. A. Flemming (8) and Sadovsky (32) have described, in addition, cells with the chromatic substance massed about a centrally placed nucleus, as in Plate IV, Fig. 17.

From the character of the alteration in the motor cells and dorsal root ganglion, and its correspondence with the changes due to injury to the peripheral nerves, it seemed desirable to search outside of the nerve cells and cord for the primary lesion. This resemblance, coincident in both localities, cord and ganglia, at once suggested examination of the peripheral nerves. Unfortunately this was not done in the three human cases* nor in the first three rabbits or Rabbit VI.

THE PERIPHERAL NERVES IN EXPERIMENTAL CASES.

As the lumbar portion of the cord in all cases seemed chiefly involved the sciatic nerves were the ones excised, with some muscular connections, and in Rabbit V in connection with the cord.

Some parts of these were examined fresh, teased in glycerine, and stained with osmic acid. Other portions were hardened in Müller's fluid, and subsequently stained by Marchi's and Weigert's methods; and still others, hardened in Flemming's solution of formaline, were stained with carmine, safranin, polychrome methylene-blue, iron-hæmatoxylin and erythrosin. Some of the nerves hardened in formaline were stained by the method used by Kolosoff for heart muscle, which was found to give excellent results.

Degenerations were found in Rabbits Nos. IV, V and VII, the earliest case examined being Rabbit IV, which lived 52 hours after

* Since the completion of this paper I have examined portions of the sciatic nerve from a fatal case of human typhoid fever and have found definite parenchymatous degeneration similar to that described in the rabbit's nerves.

inoculation; numerous nerve fibres in the sciatic were found degenerated. These degenerations consisted in varicose swelling of the fibres, and breaking up of the neurokeratin and myeline sheath. In these vesicle-like spaces numerous small lymphoid cells and the shred-like remains of the structural network occur, while the axis cylinder is swollen, twisted, and no longer clear and homogeneous. About these areas the small-celled infiltration is quite noticeable.

In the later animals, especially in Rabbit VII, the altered myeline is broken up into balls and a deeply staining, granular detritus, which is collected for the most part into regular, oval or spherical bodies, suggesting the so-called "compound granular corpuscles" (Plate IV, Fig. 18). This suggestion is strengthened by the fact that, in sections hardened in formaline and Flemming's solution and stained with polychrome methylene-blue, safranin, etc., there may be seen lying within these swollen, degenerated areas, in addition to small lymphoid cells, large swollen cells with rather pale vesicular nuclei, placed somewhat eccentrically, in a granular-looking protoplasm. In such nerves the axis-cylinder is broken, twisted and much enlarged, the swollen club-like extremities projecting into the vesicular spaces. In Rabbit VII there is seen about the more degenerate spots a proliferation of the nuclei of the sheath of Schwann and of the interstitial tissue.

These lesions are especially marked in the gluteal, and some other lower branches of the sciatic nerve, and apparent especially in the intramuscular branches. The corresponding muscles are, in Rabbits V and VII, more or less affected, showing a fatty degeneration, staining black with osmic acid, loss of cross striation, atrophy of fibres, with, in the latter animal at least, some proliferation of the interstitial tissue.

Sections of the cord in Rabbits IV and VII were examined by Marchi's and the Pal-Weigert method, both in transverse and longitudinal section. A few nerve fibres, scattered indefinitely through the white substance, showed changes pointing to some slight degeneration of the myeline sheath but so slight in comparison with that of the peripheral nerves that they do not seem of much importance.

Marked degenerations are essentially peripheral and are such as are found in typical neuritis of the parenchymatous form, and call to mind especially those described by Sidney Martin (23) in his studies on diphtheria.

It would seem as if so profound an alteration of the nerves and muscle, a true Wallerian degeneration of the former, could scarcely be produced by primary lesions of the nerve cells, of the nature we have described. Such an interpretation of an alteration so superficial and slight as the disintegration and destruction of the Nissl bodies is known to be seems out of the question. It should be remembered that, except in a few cells, the achromatic substance was not much altered. Moreover, in one of the rabbits at least, the cells were quite restored to integrity, and in another undergoing a process of active restitution.

The changes could hardly have been primary in the muscles, as in Rabbit IV these were not much altered, while the nerves were quite extensively degenerated. Again, although ascending degeneration may take place in peripheral nerves, it is a process of longer duration. We must therefore conclude that we are dealing with a condition similar in its effects to section of a peripheral nerve, that is, with reaction on the nerve cell at a distance, brought about by changes in the nerves, the direct result of the action upon the latter of the typhoid poison.

Ballet (2) and Marinesco (17, 18, 19), in the cases of peripheral neuritis examined by them, found similar changes in the nerve cells, but are unwilling to infer definitely a primary alteration in the nerves, if much time has elapsed and the degeneration in the cells is so extensive that the achromatic portion is involved. This difficulty is obviated in the experimental lesions, where the whole development of the process may be traced, and which tend to show that some reliance is to be placed upon this form of lesion as an indication of the involvement of the peripheral nerves. Indeed, the peripheral nerves would not have been examined in this instance had not the lesion found proved so constant and characteristic, and in spite of the fact that it was expected that a direct toxic lesion would be discovered.

So far all experiments involving disturbance of the function of the axone produce constantly this one characteristic lesion. The fact that by infections, poisons, the use of high temperatures, the cutting off of the circulation from the cord, etc., this lesion is at times produced, along with other changes, proves nothing against its specificity, as all these injurious conditions might act as well directly on the peripheral nerves as on the cells. It is also held that in any morbid condition the disturbance of function of any one or all organs of the body produces abnormal conditions, which would mask the specific effect of the infectious agent. But it is well known that all organs or the same organs are not affected alike in the course of the same infection in different individuals, and hence such conditions and the lesions they might produce would not be constant. The lesions found in typhoid fever are constant. Therefore, where the lesion in question is predominant and constant it seems as if it should be referred to a peripheral effect of the infectious agent, until such effect can be proved to be excluded by examination of the peripheral nerves. The exclusion of these, however, is not an easy problem, as it has been shown by Nissl (26) that even such slight disturbance as contact of nerves with a grain of salt is sufficient to produce reaction upon the cells. Nissl also observed these cell alterations 6 hours after removal of part of the nerve, while Dutil (2) first notes degeneration in the proximal nerve stump by Marchi's method in 37 days. Thus an area of injury might be overlooked.

At least in these cases of typhoid infection it would seem that the toxine acts primarily upon the peripheral portion of the neurone, so far as this can be affected more in one portion than another, and there seems to be no reason why a unit which differs structurally in its parts should not differ as to these in its vulnerability. This means that the typhoid toxine is a powerful peripheral nerve poison, and that its effect is felt early and continuously, although it may be so slight as not to become manifest clinically, or be so slightly manifested as to be readily overlooked under existing means of observation in the height of the disease. Thus a slight loss of muscular control might well be veiled. At times, however, some complaint on the part of the

patient attracts the attention of the physician, and the condition is recognized. Such, for example, is probably the condition of "tender toes" not uncommon at this stage of the disease, as suggested by Dr. Osler in Vol. V of the Johns Hopkins Hospital Reports. Should the lesion be of a grave nature and produce a paralysis lasting into convalescence, then for the first time when the patient attempts to use these muscles, the true condition becomes apparent, and "develops" as one of the many varieties of post-typhoid neuritis. Among these occurs, though rarely, a diplegia, which is very resistant and slow to improve. By some this is, for clinical reasons, held to be a primary degeneration of the nerve cells, a true poliomyelitis. In two of the rabbits the same phenomenon developed but was due evidently to a peripheral nerve lesion, and it is not impossible that some of these nerves may fail to recover their peripheral connection, so that there ensues a complete degeneration of their appropriate cells (Flatau, 5; Marinesco, 18).

The aim of this investigation, the determination of the Nissl changes in typhoid infection, and, if found to be uniform, the assignment of some cause for this uniformity, is thus attained. I desire to express my thanks for valuable advice and assistance to Professors Welch, Flexner and Barker.*

Conclusions.—(1) The application of the Nissl method to the study of the motor cells of the spinal cord, and the nerve cells of the dorsal root ganglia in typhoid fever, shows that these cells regularly suffer pathological changes in the course of the infection.

(2) The alterations in the motor cells are more constant and of a severer grade than are those in the cells of the sensory ganglia. The more characteristic changes consist of disintegration, solution and destruction of the chromatic substance of the cell starting from the

* Since writing the above, a thesis by C. Voinot, delivered at Nancy, July 31, 1897, and entitled "Recherches anatomo-pathologiques sur la moelle épinière," fell into my hands. Of twelve cases of typhoid fever among other infections, five were examined by Nissl's method, and evidently with results much as above. The author, however, seems to lay stress upon alterations, perhaps definite, but slight, found in the cord and root fibres, the peripheral nerves not having been examined, and concludes that this disease belongs, with the others described, in the category of acute infectious myelitides, and that the alteration of the cells is coincident merely.

axone hillock and proceeding toward the nucleus. Coincidently the nuclei of the affected cells seek the periphery. Alterations are also suffered by the nucleus and nucleolus.

(3) While this central form of chromatolysis is the prevailing type of pathological change, disintegration, etc., of the Nissl bodies situated in the periphery of the cell and in the dendrites is also observed (peripheral chromatolysis).

(4) In experimental infection with typhoid bacilli in rabbits a similar series of lesions in the corresponding nerve cells in the spinal cord and ganglia is encountered.

(5) The main or central type of lesions discovered is identical with that found in man and animals after section, destruction, or even slight injury of the peripheral nerves.

(6) The examination of the peripheral nerves arising from the lumbar segment of the cord (the site in man and rabbit of the most profound changes) in rabbits inoculated with typhoid bacilli showed well-marked evidences of parenchymatous degeneration.

(7) It is probable that lesions of the peripheral nerves in typhoid fever in human beings are common and that the post-typhoid hyperæsthesias and paralyses are due to this cause.

(8) Restitution of the chromatic granules may take place in the affected nerve cells, the new formation beginning about the nucleus and extending through the protoplasm.

DESCRIPTION OF PLATES II–IV.

All figures were drawn with a Zeiss drawing apparatus; Figs. 3, 4 and 5 with Zeiss apochr. obj. 3 mm., oc. 4; the rest (except Fig. 18) with Zeiss apochr. oil immers. 2 mm., oc. 4.

[Measurements given are the longest and shortest diameters.]

Plate II.

Fig. 1.—First human case: Cell from ventral horn, lumbar region, showing increase in size, characteristic form of chromatolysis, fine deposit in nucleus, vacuolated swollen nucleolus, and absence of chromatic substance in dendrites.

Size: cell, 78x36 μ; nucl. 24x18 μ; nucleol. 6x6 μ.

Fig. 2.—Similar to Fig. 1, showing also extension of process from axone hillock and slight dislocation of nucleus. Size: cell, 64x34 μ; nucl. 20x15 μ; nucleol. 7x7 μ.

Fig. 3.—Third human case; cell from ventral horn showing progression of the lesion shown in Figs. 1 and 2. Extreme eccentric position of nucleus with crumpled membrane and chromatic granules arranged along cellular margin. Nucleolus swollen but not much altered.

Size: cell, 63x33 μ; nucleol. 6x6 μ.

Fig. 4.—Similar to Fig. 3. Shows bulging of nucleus and extrusion (?) of nucleolus.

Size: cell, 75x15 μ.

Fig. 5.—Similar to Figs. 3 and 4. Somewhat more extensive chromatolysis, and swelling of cell.

Size: cell, 75x15 μ.

Fig. 6.—Rabbit III. Ventral horn cell. Indefinite "toxic" type of alteration; one small vacuole is shown containing a small oval body, resembling a bacillus.

Size: cell, 70x24 μ; nucleol. 4x4 μ.

Plate III.

Fig. 7.—Rabbit II. Characteristic central lesion in earlier stage. Central chromatolysis beginning about axone hillock, and spreading toward nucleus, which is slightly eccentric. On the opposite side the Nissl bodies are swollen and disintegrating.

Size: cell, 49x32 μ; nucl. 18x12 μ; nucleol. 4x4 μ.

Fig. 8.—Rabbit IV. Same lesion further advanced. As yet not much eccentricity in nucleus. Compare with Figs. 1 and 2.

Fig. 9.—(Rabbit paralyzed.) Great enlargement of cell, and destruction of chromatic substance. Loss of processes. Eccentric nucleus. Disintegrating nucleolus. [Note double nucleoli not uncommon in rabbits.] (Compare with Fig. 4.)

Size: cell, 100x36 μ; nucl., 28x14 μ; nucleol., 5x4 μ.

Fig. 10.—Rabbit V. Type of large cells in ventral horn found above entrance of sciatic, and apparently nearly restored to integrity.

Size: cell, 90x60 μ; nucl. 26x20 μ; nucleol. 6x6 μ.

Fig. 11.—Rabbit VII. Type of cell of ventral horn in altered area apparently showing first stages of regeneration. (Compare with Figs. 8 and 9.)

Size: cell, 62x82 μ; nucl. 20x20 μ; nucleol. 5x5 μ.

Fig. 12.—Second human case; cell from lumbar dorsal root ganglion. Destruction of chromatic substance and eccentric nucleus.

Size: cell, 56x40 μ; nucleus, 16x10 μ; nucleolus, 5x6 μ.

Plate IV.

Fig. 13.—Rabbit V. Similar to Fig. 14. Cell from lumbar dorsal root ganglion.

Fig. 14. Rabbit IV. Shows destruction of chromatic substance in two areas in cell body. Lumbar dorsal root ganglion.

Fig. 15.—Rabbit VII (paralyzed). Remains of chromatic substance scattered as large shreds through body of cell. Bulging of nucleus from periphery. Lower lumbar ganglion.

Fig. 16.—Rabbit VI (paralyzed). Clear zone separating peripheral collections of granules. Exaggeration of normal condition (?). Lumbar dorsal root ganglion.

Fig. 17.—Rabbit VII (paralyzed). Massing of chromatic substance about nucleus. Condition found in a number of cells in Rabbits V, VI and VII, but not always so well marked. Lowest lumbar root ganglion.

Fig. 18.—Rabbit VII. Sciatic nerve, hardened in Müller's fluid, stained by Marchi's method. Myeline sheath broken up, and detritus contained for the most part in granular cells. Zeiss objective D, ocular 4.

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FIG. 1.

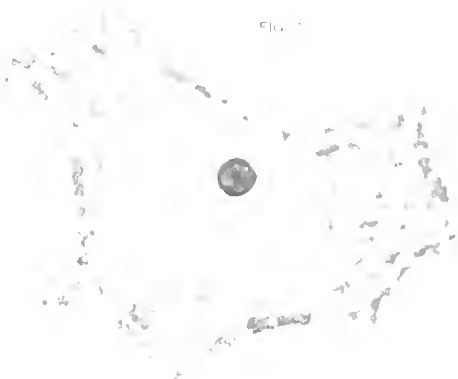


FIG. 4.

FIG. 3.

FIG. 5.

FIG. 6.

FIG. 8.

FIG. 7.

FIG. 9

FIG. 10.

FIG. 11.

FIG. 12.

FIG. 13.

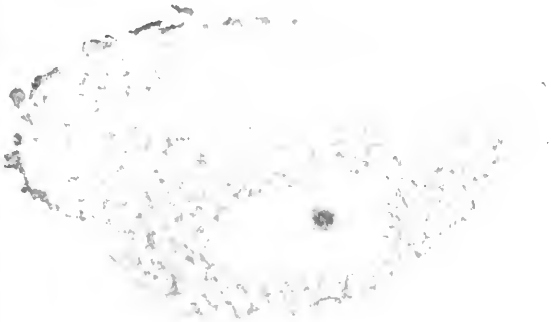


FIG. 17.

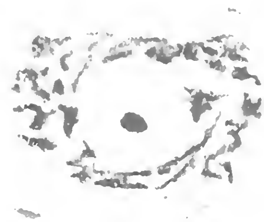


FIG. 15.

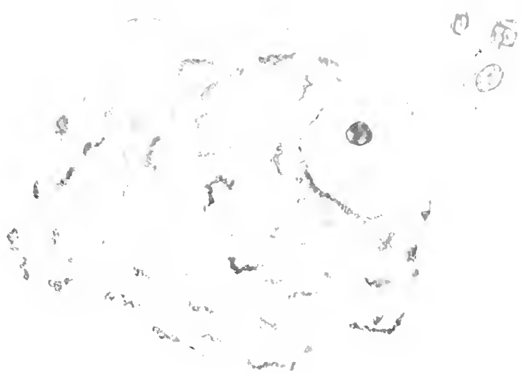


FIG. 18.

FIG. 16.

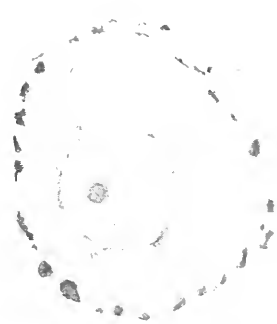
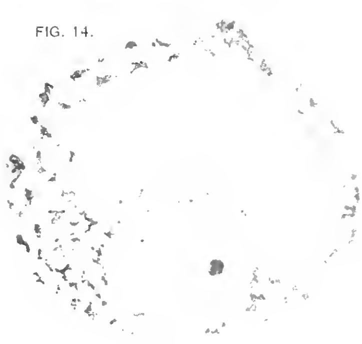


FIG. 14.



THE THERMAL DEATH-POINT OF TUBERCLE BACILLI IN MILK AND SOME OTHER FLUIDS.

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In the comparative study of tubercle bacilli from various sources recently published,* an experiment was made to learn whether any differences towards heat would be manifested by cultures from man and from cattle. Pursuing the methods hitherto employed, I suspended the bacilli in milk and exposed the suspensions at 60° C. for different periods of time. The result was at variance with current notions of the resistance of tubercle bacilli at this temperature. A second trial gave a result quite at variance with the first, so that a third trial was made. Quite unintentionally I was drawn into a series of experiments extending over a period of more than 18 months, which have yielded several facts not anticipated, and destined to change somewhat our conceptions of the heat-resisting power of tubercle bacilli. Before detailing the tests, a brief review of the experiments of others will aid us in appreciating the necessity for the present work. In all my tests I restricted myself to the temperature of 60° C. in order to keep the work within certain narrow limits. I shall therefore confine my review to the determinations of others at that temperature.

Sternberg† in 1887 inoculated a guinea-pig with sputum exposed at 60° C. for 10 minutes. The animal remained unaffected.

Yersin‡ in 1888 tested an old glycerine bouillon culture in which the

* Theobald Smith: A Comparative Study of Bovine Tubercle Bacilli and of Human Bacilli from Sputum, JOURNAL OF EXPERIMENTAL MEDICINE, 1898, iii, 451.

† Sternberg, Disinfection and Disinfectants, etc., by the Committee on Disinfectants, appointed by The American Public Health Association, p. 148. Concord, N. H., 1888.

‡ Yersin, *Annales de l'Institut Pasteur*, 1888, ii, 60.

bacilli appeared "sporulating." Heated for 10 minutes at a temperature of 60° C. in small, sealed tubes, they still multiplied and produced disease in rabbits.

Grancher and Ledoux-Lebard* found avian cultures dead after an exposure of 20 minutes at 60° C., but not after 10 minutes. Human cultures were dead after an exposure of 15 minutes. Bonhoff† cultivated a human tubercle bacillus of unknown history in glycerinated calf's-lung bouillon. An exposure of such a culture for 20 minutes to 60° C. destroyed the bacilli.

de Man‡ in Forster's laboratory made a series of tests with tuberculous tissue. The thermal deathpoints established by him are now generally published in text-books. He used chiefly the disintegrated, semi-fluid, cheesy matter from tuberculous udders. His exposures in sealed tubes at 60° C. may be briefly summarized. After an exposure for 15, 30 and 45 minutes 2 guinea-pigs inoculated with material from each exposure became tuberculous. A repetition of this test yielded the same results. Two guinea-pigs, inoculated with material from two separate exposures for 60 minutes, remained well. Sputum exposed for 60 minutes was equally innocuous. From these results it has been generally assumed that it takes 60 minutes at 60° C. to kill tubercle bacilli.

Woodhead§ fed milk, from a tuberculous udder, which had been exposed for 15 minutes to 60° C. to 6 guinea-pigs without any effect. Similarly, milk heated for 30 minutes had no effect on 5 guinea-pigs fed with it. On the other hand, milk heated 15 minutes at 60° C. and injected into the peritoneal cavity produced tuberculosis in 2 of 3 guinea-pigs. Of three guinea-pigs inoculated in the same way with milk heated 30 minutes one became tuberculous. In another series of experiments with milk from a tuberculous udder no disease resulted from milk heated 25 or more minutes. Milk heated for shorter periods was not injected. Experiments were also made with milk to which ground and minced tuberculous tissue had been added. In such milk artificially infected, the tubercle bacilli were in some instances still alive after two hours' exposure.

This brief survey shows that these various tests were made under such different conditions that they cannot be utilized to define the thermal deathpoint of tubercle bacilli even if the results were fairly

* Grancher and Ledoux-Lebard, *Arch. de méd. expér.*, 1892, iv, 1.

† Bonhoff, *Hygienische Rundschau*, 1892, ii, 1009.

‡ de Man, *Arch. f. Hygiene*, 1893, xviii, 133.

§ Woodhead, Report of the Royal Commission on Tuberculosis, 1895, p. 146.

concordant. There was, therefore, ample justification for a repetition of these experiments with cultures of known history.

Milk was at first used exclusively as the suspending fluid for obvious reasons. Later on it became necessary to use simpler fluids, such as bouillon, distilled water and physiological salt solution to clear up the discordant results obtained with milk. The manipulations were modified from one test to another either to remove some supposed defect of former methods or to answer certain new queries which former tests had raised. The cultures used were all isolated by me and their history is given in a former publication.* Blood serum from the dog set at 75° to 76° C. was used exclusively as the culture medium. The suspensions were made by rubbing masses of bacilli against the inner surface of sterile test-tubes near the bottom until a fairly homogeneous coating had been formed. The rubbing was done with a heavy platinum wire beaten into the form of a slender spatula. The suspending fluid was then poured into the tube and thoroughly stirred. The resulting suspension still contained many clumps of bacilli made up of a few to 30 or more rods. The tubes thus prepared were exposed in a water-bath (Experiments I, II), or else other tubes were used to which definite quantities of the original suspension had been added. In all cases the suspensions were injected directly into the peritoneal cavity of guinea-pigs in order to give the bacilli the best opportunity for multiplication. Further details are given in the following account of the tests described in the order in which they were made.

Experiment I. June 4, 1897. Bovine culture II and sputum culture II were used. Growth of former a thin layer like ground glass in appearance; growth of latter much richer. Suspensions made, as described, in tubes of moderately heavy glass having an internal diameter of 15 to 18 mm. and provided with a glass cap ground to the tube and having a narrow ventilating tube above plugged with glass wool. This kind of tube differs from ordinary test-tubes simply in having more limited ventilation. About 10 cc. of milk, previously heated to 60° C., was added with a pipette and the suspension gently stirred. The tubes were then clamped in a water-bath kept at 60° C. with occasional fluc-

* Loc. cit.

tuations of 0.5° above or below this point. From each tube about 2 cc. of milk were removed with a sterile pipette at the end of 30, 45, 60 and 75 minutes. Each time the suspension was gently stirred by forcing it back from the pipette to counteract the settling of the bacilli. Great care was taken not to soil the walls of the tube above the level of the fluid. The tubes themselves were immersed to a depth of about 10 cm., the surface of the suspension being about 5 cm. below the surface of the water-bath.

The vitality of the bacilli was tested by injecting about 1 cc. into the peritoneal cavity of guinea-pigs. Further details are given in Table I.

TABLE I (EXP. I).—SUSPENSIONS IN MILK.

Designation of culture.	Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes. Initial.	Final.	Result.	Remarks.
Bovine II	30 minutes.	1	548	565	—	Chloroformed in 18 days; 6 to 8 nodules on omentum.
	45 "	1	480	514	—	Chloroformed in 39 days; several nodules on omentum.
	60 "	1	435	415	—	Chloroformed in 26 days; several nodules on omentum.
	75 "	1	375	445	—	Chloroformed in 26 days; several nodules on omentum.
Sputum II	30 "	1	448	471	—	Chloroformed in 39 days; no lesions.
	45 "	1	412	474	—	Chloroformed in 43 days; 6 nodules on omentum.
	60 "	1	415	462	—	Chloroformed in 43 days; 10 nodules on omentum.
	75 "	1	375	350	—	Chloroformed in 26 days; 6 nodules on omentum.

In this first test no inoculations were made with unheated suspensions, as it was supposed that if it takes 60 minutes to kill tubercle bacilli at 60° C. the animals receiving the milk heated 30 minutes would be equivalent to control animals. This, however, failed to be the case, as all animals remained free from disease. This unexpected result suggested the possibility that the cultures may have been dead when used. An examination of the culture records showed, however, that subcultures from the cultures used in the experiment were fertile. There was nothing in the appearance of the cultures, only 11 days old, to suggest loss of vitality.

Experiment II. June 25, 1897. Sputum culture III and swine culture I used. Both 13 days old. Growth of sputum culture rich, that

of the other less so. The procedure differed from that of Experiment I in that the milk was added cold to the smeared tube. Due allowance for the warming of the fluid is made in the general summary given in Table IX (p. 230). Coverslip preparations showed the swine-culture suspension to be denser than the other. The inoculations are given in Table II.

TABLE II (EXP. II).—SUSPENSIONS IN MILK.

Designation of culture.	Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes. Initial.	Final.	Result.	Remarks.
Sputum III	0 minutes.	1	555	260	+	Dying on 27th day; chloroformed. General tuberculosis.
	15 "	1	520	545	+	Chloroformed in 46 days; lesions in omentum, lymph glands, spleen, liver.
	30 "	1	475	493	—	Chloroformed in 47 days; about a dozen nodules on omentum.
	45 "	1	437	527	+	Chloroformed in 55 days; nodules and larger necrotic foci on omentum. Tuberculous retrogastric gland; minute yellow spots in liver. Thoracic glands enlarged but free from necroses.
Swine I	0 "	1	600	..	+	Dies in 13 days. Usual acute tuberculosis following abdominal injection.
	15 "	1	550	570	+	Chloroformed in 45 days. Tuberculosis of all thoracic and abdominal glands. A few foci in spleen and liver.
	30 "	1	482	492	—	Chloroformed in 47 days. Some nodules on omentum.
	45 "	1	422	425	+	Chloroformed in 55 days. General tuberculosis.

The outcome of this experiment was quite different from that of the preceding. Only the animals which received the milk suspension heated 30 minutes may be said to have received no living virulent bacilli. Those receiving milk heated 15 and 45 minutes from both cultures were distinctly tuberculous.

Experiment III. This may be disposed of briefly as it adds nothing new to the information gained by the other tests. It is, however, of

some general interest. July 1, 1897. The udder of a cow of which one-quarter was enormously enlarged by tuberculosis and the others atrophied and giving but little milk was the starting point. The tuberculous tissue was largely broken down into a caseous, diffluent mass. From one atrophied quarter a little milk was obtained with the hope that the thermal deathpoint of tubercle bacilli naturally suspended in that fluid might be obtained as a check upon the artificial suspensions. Though tubercle bacilli were not found in it with the microscope still the inoculations were made; 2 cc. of milk heated 0, 20, 35, 50 and 65 minutes were injected, each lot into 2 guinea-pigs. Killed from 6 to 9 weeks after inoculation, they were found free from disease. The milk was probably free from tubercle bacilli and the disease strictly limited to one-quarter.

Experiment IV. This test with bovine caseous material suspended in ordinary peptone bouillon was made mainly to determine whether the tubercle bacillus might exist in some more heat-resisting stage in caseous masses in which multiplication seems to be at a standstill.

TABLE III (EXP. IV).—BOUILLON SUSPENSIONS OF CASEOUS MATTER (CATTLE).

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.6	342	404	+	Chloroformed in 42 days. General tuberculosis.
0 "	2.	482	484	+	Chloroformed in 27 days. General tuberculosis.
0 "	2.	657	640	+	Chloroformed in 39 days. General tuberculosis.
20 "	"	646	693	—	Chloroformed in 31 days. One nodule on omentum.
" "	"	383	505*	—	Chloroformed in 55 days. No lesions.
35 "	"	445	622	—	Chloroformed in 54 days. No lesions.
" "	"	612	701	—	Chloroformed in 31 days. No lesions.
50 "	"	485	593†	—	Chloroformed in 106 days. No lesions.
" "	"	339	445	—	Chloroformed in 31 days. One nodule on omentum.
65 "	"	567	592†	—	Chloroformed in 129 days. One nodule on omentum.
" "	"	329	418	—	Chloroformed in 18 days. No lesions.

* Pregnant when chloroformed.
† Had given birth to a litter of healthy young 4–6 weeks before it was chloroformed.

Nov. 26, 1897. Completely caseous matter from the lungs of a cow was scraped into a small dish and kept on ice until next day. It was

ground in a sterile mortar with bouillon and the turbid fluid passed through several thicknesses of linen. In the densely turbid, pale yellowish fluid, consisting microscopically of fatty debris chiefly, tubercle bacilli were not found. 7 cc. were carefully transferred with a pipette to a number of test-tubes with ground glass cap. These were immersed in the 60° bath so that the surface of the fluid was 5 cm. below the level of the bath. A tube was removed at the end of 20, 35, 50 and 65 minutes. Table III (p. 222) shows that even 20 minutes was sufficient to destroy the bacilli, and that in caseous tissue tubercle bacilli do not possess any greater power of resistance to heat than in cultures on serum.

TABLE IV (EXP. V).—A. SUSPENSION OF BOVINE BACILLI IN BOUILLON.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes. Initial.	Final.	Result.	Remarks.
0 minutes.	0.5	581	468	+	Dies in 17 days. Acute tuberculosis.
20 "	1.5	544	582	—	Chloroformed in 52 days; a dozen nodules on omentum.
(Bacilli from nodules of preceding case.)		340	405	—	Chloroformed in 37 days; no lesions.
35 minutes.	1.5	554	638	—	Chloroformed in 43 days; a dozen nodules on omentum, slight hyperplasia of lymph glands.
50 "	"	528	607	—	Chloroformed in 49 days; six nodules on omentum.
65 "	"	349	498	—	Chloroformed in 58 days; nodules on omentum, one in ovarian ligament.

B. SUSPENSIONS IN MILK.

Time of exposure to 60° C.	Quantity injected in cc.	Initial.	Final.	Result.	Remarks.
(Milk not infected.)	3.5	421	..	—	Dies in 7 days of streptococcus infection; abscesses in omentum and between spleen and liver.
(Milk not infected.)	"	401	..	—	Dies in 7 days of streptococcus infection.
0 minutes.	1.5	591	430	+	Dies in 15 days; acute tuberculosis.
20 "	"	401	478	+	Chloroformed in 52 days. General tuberculosis.
35 "	"	510	671	+	Chloroformed in 43 days. Tuberculosis of lymph glands; slight infection of liver and spleen.
50 "	"	401	514	+	Chloroformed in 49 days. General tuberculosis.
65 "	"	369	510	+	Chloroformed in 58 days. General tuberculosis.

Experiment V. The preceding tests taken together suggested that tubercle bacilli may be much more resistant to heat in milk than in

bouillon. This suggestion was tested in the following manner with bovine culture III:

A. Bouillon series, Feb. 15, 1898. The culture, 17 days old, was suspended in bouillon having a reaction equivalent to 1.45 per cent. of a normal acid solution (phenolphtalein). Ordinary, cotton-plugged thin-walled test-tubes were used, 15 to 18 mm. in diameter and 15 cm. long. Each tube received 2 cc. of the suspension and was immersed to a depth of 10 cm. in the bath. One was removed at the end of 20, 35, 50 and 65 minutes.

B. Milk series. The tubes were prepared in the same way. The milk was about 6 hours old, kept in the refrigerator. Acidity 1.9 per cent.

The result of the inoculations given in Table IV (p. 223) shows that the bacilli in the bouillon suspensions had been killed, while some of those in the various milk tubes had survived.

TABLE V (EXP. VI).—A. SUSPENSION OF BOVINE BACILLI IN WATER.

Time of exposure at 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
15 minutes.	1.5	304	548	—	Killed in 64 days. Some nodules on omentum.
25 "	"	358	416	—	Killed in 35 days. Some nodules on omentum.
B. SUSPENSION IN MILK.					
(Milk not infected.)	3.5	732	..		Dies in 6 days. Abscesses in abdominal cavity. Streptococcus infection.
(Milk not infected.)	"	379	350	—	Chloroformed in 31 days. Large abscess in abdominal cavity due to minute bacilli.
(Milk not infected.)	"	444	591	—	Chloroformed in 59 days. No lesions. Milk from same dairy but from a lot 11 days later than preceding.
0 minutes.	1.	342	..	+	Dies in 9 days. Acute tuberculosis and streptococcus infection.
20 "	1.4	317	360	—	Killed in 61 days. Several nodules on omentum.
35 "	2.	409	411	+	Killed in 29 days. Tuberculosis of omentum, peritoneum and lymph glands.
50 "	1.5	363	453	—	Killed in 64 days. Several nodules on omentum.
65 "	2	370	508	+	Killed in 64 days. Slight tuberculosis of lymph glands of abdomen, liver and spleen.
80 "	2	420	595	—	Killed in 64 days. A few nodules on omentum, 2 minute ones on testicles.

Experiment VI. April 28, 1898. This is an exact counterpart of Experiment V with certain minor modifications. The culture is a young (seven-day) culture of bovine bacillus IV. The suspensions were made, as before, in milk, and in distilled water. They were exposed in cotton-plugged test-tubes. The ready destruction of the bacilli in water, their irregularly persistent vitality in the milk is again demonstrated in Table V (p. 224). Cultures from the water suspensions confirmed the inoculation results. The control unheated suspension gave rise to a vigorous culture on dog's serum, the cultures from the heated suspensions remained sterile.*

TABLE VI (EXP. VII).—A. SUSPENSION OF BOVINE BACILLI IN WATER.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	..	..	..	+	Culture positive.
12 "	1.5	406	474	—	Culture negative. Animal killed in 75 days. Slight hyperplasia of mesenteric and sternal glands. Several nodules on omentum and one on abdominal wall.
22 "	1.5	366	490	—	Culture negative. Animal killed in 56 days. Some nodules on omentum.
B. SUSPENSION IN MILK.					
(Milk not infected.)	2.0	384	664	—	Chloroformed in 75 days. No lesions.
0 minutes.	0.5	375	270	+	Dies in 32 days. General tuberculosis. Abscess in omentum containing cocci.
17 "	1.5	367	448	—	Killed in 56 days. Some nodules on omentum.
32 "	"	353	443	—	Killed in 56 days. Some nodules on omentum.
47 "	"	403	451	—	Killed in 56 days. Some nodules on omentum.
62 "	"	385	508	—	Killed in 56 days. Some nodules on omentum, and cheesy focus in subcutis at point of injection.
77 "	"	395	498	—	Killed in 56 days. Some nodules on omentum.

Experiment VII. May 18, 1898. The procedure differed from that of former tests in that the suspensions were exposed in com-

* Cultures from milk suspensions were not attempted because of the anticipated presence of contaminating bacteria. To sterilize the milk before use by discontinuous boiling would obviously fail to imitate natural conditions.

pletely submerged, sealed glass tubes. An old culture was used to determine if more resistant bacilli appear after a time.

Bovine IV, grown for 30 days on the same lot of dog's serum on which the young culture of the preceding experiment had grown was used. The milk had an acidity of 1.7 per cent. Parallel exposures in distilled water were also made. The tubes were made of ordinary glass tubing having an internal diameter of 6 mm. Both ends were drawn out in the flame. One end was sealed at once. About 2 cc. of the suspension was inserted through the other opening with a delicate pipette and the latter then sealed in the flame. This proved a better method than drawing the suspension up first and then sealing as it prevented the usual sputtering when the sealing is attempted and the indiscriminate distribution of tubercle bacilli. Each sealed tube contained one-half to one-third its volume of air. They were laid horizontally on a rack about 7 cm. below the surface of the bath close to the thermometer bulb. The results of the inoculation are given in Table VI (p. 225).

The outcome of this test was a surprise, for not one of the guinea-pigs inoculated with heated suspensions became tuberculous. For the water suspensions the culture test agreed with the animal test. A vigorous culture was obtained from the unheated suspension in two weeks. The other tubes were sterile after 4 weeks. It will be at once thought that the use of the sealed tubes accounts for this, but why should the suspensions in fluids other than milk give uniform results in test-tubes? The remaining two experiments, I think, give a satisfactory answer to this query.

Experiment VIII. August 12, 1898. In this test bovine culture VI was used. The bacilli were exposed in sealed tubes. The milk, kept over night in the refrigerator, had a heavy layer of cream on it next morning. It was thoroughly mixed before use. Acidity 1.5 per cent. Suspensions in normal salt solution were also made. The result of the test given below in Table VII did not differ materially from that of the preceding test. In the 12-minute tube the bacilli suspended in salt solution were not all destroyed. The guinea-pig inoculated with the milk suspension exposed for 17 minutes had a suspicious subcutaneous lymph gland with which unfortunately fresh inoculations were not made, so that this case remains in doubt. However, the lesion was so slight, if due to living bacilli, that one bacillus may have caused it.

TABLE VII (EXP. VIII).

A. SUSPENSION OF BOVINE BACILLI IN NORMAL SALT SOLUTION.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes. Initial.	Final.	Result.	Remarks.
12 minutes.	1.5	338	464	+	Chloroformed in 106 days. Tuberculosis of both testicles, of left popliteal, and of lumbar gland. All foci softened. Bronchial glands tuberculous; still firm.
22 "	"	376	508	—	Chloroformed in 106 days. Two small, softened nodules on omentum; one on testicle.

B. SUSPENSION IN MILK.

(Milk not infected.)	3.5	251	640	—	After 105 days weighs 640. Alive after $5\frac{1}{2}$ months.
0 minutes.	0.5	373	..	+	Dies in 9 days of acute tuberculosis.
17 "	1.5	388	494	+	Killed in 57 days. Slightly enlarged and partly necrotic popliteal gland.
32 "	"	398	532	—	Killed in 57 days. Several 2–3 mm. nodules on omentum.
47 "	"	366	545	—	Killed in 63 days. No lesions.
62 "	"	298	423	—	Killed in 63 days. Several nodules on omentum.
77 "	"	267	479	—	Killed in 63 days. Slightly conspicuous retrogastric and thoracic lymph glands.

Experiment IX. Nov. 17, 1898. In this test bovine culture III, used in Experiment V, was again employed. The suspensions were prepared from a vigorous culture 13 days old. Its vitality was demonstrated by the rapid, rich growth of a subculture made at the same time with the suspensions. The bacilli suspended in distilled water were exposed in the test-tubes with ground glass cap, each tube receiving 2 cc. The milk suspensions were exposed both in these tubes (4 cc.) and in sealed tubes. The outcome of the tests made thus far had suggested the theory that the occasional survival of bacilli leading to the irregular results tabulated might be due to the formation of the surface pellicle into which the bacilli are carried by particles of fat. Here their thermal deathpoint might be higher than when submerged in a watery fluid. Hence the pellicle which had formed during the exposure in the test-tubes was in part removed with a platinum loop and stirred in with the rest of the milk to be injected. The milk used had a layer of cream (formed over night) equivalent to 14 per cent. of the whole column. Acidity 1.67 per cent. The bore of the sealed tubes was 4 mm. They contained about 1.5 cc. milk and some air. Coverslip preparations dem-

onstrated the presence of large numbers of bacilli chiefly in clumps. The results are summarized in Table VIII.

TABLE VIII (EXP. IX).—A. SUSPENSION OF BOVINE BACILLI IN DISTILLED WATER EXPOSED IN CULTURE TUBES WITH GLASS CAP.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes. Initial.	Final.	Result.	Remarks.
0 minutes.	0.5	384	..	+	Dies in 25 days of tuberculosis. Suspension made from the same culture 12 days after the general experiment to replace milk controls (see below).
10 "	1.5	332	418	+	Killed in 62 days. Tuberculosis of lymph nodes, liver, spleen, peritoneum.
15 "	1.5	372	438	+	Killed in 61 days. Tuberculosis of lymph nodes chiefly.
20 "	1.5	302	331	+	Killed in 61 days. Generalized tuberculosis.

B. SUSPENSIONS IN MILK IN SEALED PIPETTES.

Time of exposure to 60° C.	Quantity injected in cc.	Initial.	Final.	Result.	Remarks.
(Milk not infected.)	4.0	524	..		Dies in 48 hours of streptococcus peritonitis and septicæmia.
0 minutes (control).	0.5	537	..	+	Dies in 6 days of streptococcus infection and tuberculosis (?).
7 minutes.	1.5	450	500	+	Killed in 62 days. Tuberculosis of lymph nodes. Slight infection of liver and spleen.
12 "	1.5	441	503	+	Killed in 62 days. Slight disease of lymph nodes. Tuberculosis of left scrotal sac.
17 "	0.5	491	605	—	Killed in 62 days. Slight localized thickening of omentum.
32 "	1.5	354	..	—	Dies in 15 days.* Six or seven small nodules on omentum; 2 on mesocolon.
47 "	1.5	368	492	—	Killed in 62 days. Several softened nodules on omentum.
62 "	1.5	303	330	—	Killed in 62 days. Some local thickenings of omentum.

C. SUSPENSIONS IN MILK IN CULTURE TUBES AS IN A.

Time of exposure to 60° C.	Quantity injected in cc.	Initial.	Final.	Result.	Remarks.
20 minutes.	1.5	378	275	+	Dies in 38 days. General tuberculosis.
35 "	1.5	272	291†	+	Dies in 52 days. General tuberculosis.

* Diphtheritic paralysis. Had been used for antitoxin test 24 days before inoculation. Only a slight transient swelling followed.

† Weight 10 days before death.

This test strengthens the supposition that the pellicle which forms on milk in test-tubes during heating is responsible for the increased resistance of milk suspensions of tubercle bacilli and the irregular

results hitherto obtained.* The two guinea-pigs inoculated with milk exposed 15† and 30† minutes together with some of the pellicle died of tuberculosis in 38 and 52 days respectively, while two other guinea-pigs inoculated with suspensions in milk and water exposed only 5† minutes were found with but slight lesions after 60 days.

A summary of all the tests made is given in Table IX (p. 230), together with some additional data concerning the cultures used. The figures in parentheses following the figures giving the total exposure at 60° C. are an approximate correction representing the actual time of exposure at that temperature.

A brief explanation of the tabulated autopsy notes of the guinea-pigs seems necessary in view of the fact that the conclusions to be drawn rest chiefly on them. It will be observed from the tables that in many cases small nodules were found on the omentum without any traces of tuberculosis in other organs, even the nearest lymph nodes. These nodules were studied histologically at different stages, although it was manifest from the purely local character of the lesions and the knowledge already gained from the researches of Prudden and Hodenpyl and of Straus concerning the action of dead tubercle bacilli that these nodules were the product of dead bacilli swept together into a mass in some way on different places on the omentum. It would be beyond the scope of this article to go into minute details concerning the structure of these nodules. Suffice it to say that within two to four weeks after the intra-peritoneal injection they are one to three mm. in diameter and translucent in appearance. They consist, then, of epithelioid and lymphoid cells, among which are many nuclear figures. In this stage the mass may contain roundish cell groups closely resembling the "germinative centres" of follicles in lymph nodes. In later stages the larger nodules undergo softening centrally, and the creamy-white mass there formed contains readily

* This pellicle, which is familiar to all who have scalded milk, is a feebly cohesive mass easily washed out in patches for microscopic examination by diluting the heated milk in water. The patches consist of fat globules and an amorphous, cohesive substance of slight refrangibility, by which they are evidently held together.

† These figures are corrected by subtracting the time required to bring the fluid to 59.5° C.

TABLE IX.—SUMMARY OF EXPERIMENTS.

Source of bacilli.	Total period of cultivation on dog's serum.	Age of culture used.	Nature of suspending fluid.	Tubes used.	Result. (The figures signify minutes, the sign + tuberculosis, the sign — no tuberculosis.)
Caseous material from cow's lung.	......	....	Peptone bouillon	Culture tubes with glass cap	—20(15), —35(30), —50(45), —65(60)
Bovine culture III	9 mos. 27 days	17 days	Peptone bouillon	Test-tubes with cotton plug	—20(15), —35(30), —50(45), —65(60)
Bovine culture IV	11 mos. 26 days	7 days	Distilled water	Test-tubes with cotton plug	—15(10), —25(20)
Bovine culture IV	13 mos.	30 days	Distilled water	Sealed glass tubes	—12(10), —22(20)
Bovine culture VI	7 mos. 19 days	10 days	Normal salt sol.	Sealed glass tubes	+12(10), —22(20)
Bovine culture III	18 mos. 29 days	13 days	Distilled water	Culture tubes with glass cap	+10(5), +15(10), +20(15)
Bovine culture II	6 mos. 7 days	11 days	Milk	Culture tubes with glass cap.	—30, —45, —60, —75
Sputum culture II	6 mos. 14 days	11 days	Milk	Culture tubes with glass cap	—30, —45, —60, —75
Sputum culture III	4 mos. 10 days	13 days	Milk	Culture tubes with glass cap	+15(10), —30(25), +45(40)
Swine culture I	13 mos. 17 days	13 days	Milk	Culture tubes with glass cap	+15(10), —30(25), +45(40)
Bovine culture III	9 mos. 27 days	17 days	Milk	Test-tubes with cotton plug	+20(15), +35(30), +50(45), +65(60)
Bovine culture IV	11 mos. 26 days	7 days	Milk	Test-tubes with cotton plug	—20(15), +35(30), —50(45), +65(60)
Bovine culture IV	13 mos. 26 days	30 days	Milk	Sealed glass tubes	—17(15), —32(30), —47(45), —62(60), —77(75)
Bovine culture VI	7 mos. 19 days	10 days	Milk	Sealed glass tubes	+17(15), —32(30), —47(45), —62(60), —77(75)
Bovine culture III	18 mos. 29 days	13 days	Milk	Sealed glass tubes	+(75), +12(10), —17(15), —32(30), —47(45), —62(60)
Bovine culture III	18 mos. 29 days	13 days	Milk	Culture tubes with glass cap	+20(15), +35(30)

demonstrable tubercle bacilli. The softening is essentially a process of caseation, never of suppuration. The necrosis is sharply demarcated from the enveloping dense outer layer of the nodule and thereby differs clearly from the progressive peripheral invasion of living bacilli. It is not possible, however, to make trenchant distinctions between early lesions due to living and to dead bacilli, and so far the progressive metastatic nature of the disease as recognized with the unaided eye seems to me the most satisfactory guide, provided a sufficient period of time (at least 50 days) be allowed to elapse between the inoculation and the examination of the animal. In but one instance (Experiment V) the bacilli from a softened focus due to dead bacilli were injected into a guinea-pig. The result was negative.

There is nothing of importance to be recorded concerning the macroscopic characters of the inoculation disease in guinea-pigs. Its protean character, as exemplified by the diverse localizations, makes a very thorough examination of every animal essential. In general, the retarded disease has its chief seat in the lymph nodes, which may attain a large size. This is particularly true of the retrogastric and lumbar, and of the bronchial and neighboring sternal nodes, whose large dimensions with visibly intact lungs suggest the bovine type of disease. In several cases the testicles and surrounding scrotal tissue were involved precisely as in glanders (following either subcutaneous or intro-abdominal inoculation). It is probable that in these experiments male guinea-pigs would be preferable, since the disease may be recognizable during life. In no case was there any evidence of air infection. In fact, I have not encountered it at any time among the animals kept for several years in the room used for these experimental animals. It is again evident from these tables that the weight of the guinea-pig is no guide in determining the presence or absence of tubercular foci.

The foregoing experiments demonstrate that tubercle bacilli are no more resistant to heat than many other bacilli not producing spores, and that at 60° C. destruction is complete in 15 to 20 minutes. Even after exposures lasting 10 minutes the bacilli were dead in most

instances. After 5-minute exposures the inoculation disease produced in guinea-pigs was greatly retarded, even though three times the control dose was injected.* When, however, milk is used as the suspending fluid, the formation of a surface pellicle into which bacilli are carried by fat globules shields them from the effect of the heat so that they may survive an exposure of 65 minutes. The peculiarly irregular results obtained by the Royal Commission† are probably to be explained in the same way. The importance of a clear understanding of this phenomenon in the pasteurization of milk is obvious, and it remains to be seen how far bottled milk may be freed from tubercle bacilli without resorting to the higher temperature of 68° C. now generally employed. Probably a complete immersion, or else a complete filling of the receptacle, may furnish the conditions desired.

These conclusions may be objected to on the ground that the tubercle bacilli may have suffered in power of resistance during cultivation, but the experiment with caseous material from cattle (IV) will, I think, silence this objection. The different results obtained by de Man, already quoted and now generally accepted, are probably due to the fact that he used caseous material or else tuberculous tissue, which he ground up in a mortar and only diluted with salt solution "when necessary." Such dense suspensions cannot be used to determine the thermal deathpoint to be compared with those of other bacilli, nor can they be regarded as imitating the conditions under which tubercle bacilli appear in milk.

The method of using narrow sealed tubes has shown itself superior to that which employs ordinary test-tubes so far as milk suspensions are concerned. When other fluids were used the results were unequivocal and in harmony with the other method, excepting, per-

* It should be borne in mind that the suspensions used were quite dense. In a small drop spread out on cover glasses, dried and stained, large numbers of bacilli singly and in clumps were seen in every field of a $\frac{1}{12}$ objective.

† Woodhead, loc. cit.

haps, in the last experiment, in which an increased resistance of the tubercle bacilli in water is manifest.*

It simply remains to point out the possibility that the resistance of other pathogenic bacteria to heat may be increased in milk. Hitherto all practical details in sterilization have been based on the thermal deathpoint of bacteria in bouillon and other fluids.

Conclusions.

1. Tubercle bacilli when suspended in distilled water, normal salt solution, bouillon and milk, are destroyed at 60° C. in 15 to 20 minutes. The larger number are destroyed in 5 to 10 minutes.

2. When tubercle bacilli are suspended in milk, the pellicle which forms during the exposure at 60° C. may contain living bacilli after 60 minutes.

* This test was repeated, after the completion of this article, for another purpose with the same culture and under the same conditions, and the same resistance to a net exposure of 15 minutes was noted. In the suspensions in water delicate floating fragments of a pellicle of bacilli were noticed which could not be submerged by vigorous shaking. Pellicles behaving similarly are frequently seen on bouillon cultures of colon and typhoid bacilli. That this phenomenon was related to the slightly increased resistance of the culture seems probable in view of the results obtained with milk suspensions in sealed pipettes. In any case those who are inclined to repeat these tests would do well to immerse completely all suspensions whether in milk or water so as to expose such floating bacilli to the same conditions of heat and moisture under which the suspended bacilli are.

THE CHANGES PRODUCED BY THE GROWTH OF BACTERIA IN THE MOLECULAR CONCENTRATION AND ELECTRICAL CONDUCTIVITY OF CULTURE MEDIA.*

By G. N. STEWART, Cleveland.

PLATES V–VIII.

By determining from time to time the freezing point of a liquid in which bacteria are growing it is possible to estimate the changes that take place in the molecular concentration.†

By determining the electrical conductivity a measure of the number of dissociated ions in unit volume of the solution may be obtained. The amount by which the freezing point is depressed below the freezing point of distilled water is directly proportional to the molecular concentration when the dissolved substances are incapable of hydrolytic dissociation, like cane sugar or proteids for instance. In the case of dissociable substances, like sodium chloride, the same is true when we reckon each ion as a molecule. The relation between the electrical conductivity and the concentration of the electrolytes is not so simple, since the specific conductivity of a solution depends not only on the number of dissociated molecules in a given volume, but also on the velocities of the ions. But there is always a relation, and when this can be established for a particular case, it can be used to calculate the concentration. For example, when only one electrolyte is present, the concentration can be at once determined from the electrical conductivity by interpolating values in a table showing the relation between the conductivity of the substance and the amount of it in solution. If more than one electrolyte be present, the electrolytes may be such as have approximately equal ionic veloc-

* This paper was read before the British Medical Association (Section of Physiology) at Edinburgh, July, 1898.

† The molecular concentration may be defined as the number of gramme-molecules of the dissolved substance in a litre of the solution.

ities. In this case the electrical conductivity can be taken as directly proportional to the total concentration of the electrolytes. If more than one electrolyte is present and the ionic velocities are widely different, then a quantitative determination of one or more of the electrolytes may be made by chemical methods, and the concentration of those that remain be deduced from the electrical conductivity. And even in complex solutions, when without actually knowing the ionic velocities we know the limits between which they must lie, we can often determine from the electrical conductivity whether the number of dissociated molecules in a given volume is increasing or diminishing, and, within certain limits of accuracy, by how much it is increasing or diminishing. This calculation can be controlled and the limits of error narrowed by taking account of the total molecular concentration (including that of the dissociated ions) deduced from observations on the freezing point. For instance, if we found that certain bacteria growing in solution of albumin caused a diminution in the freezing point without any change in the electrical conductivity, this would indicate that the large albumin molecules were being broken down into smaller, but as yet non-dissociable, molecules, possibly into peptones. If, later on, we found that while the molecular concentration, as indicated by the lowering of the freezing point, was still increasing, the electrical conductivity also began to increase, we should have to conclude that in the course of the decomposition the point had now been reached where some of the comparatively small molecules were at length dissociable. If the molecular concentration and the electrical conductivity were found both to increase from the beginning of the bacterial growth, it would be proved that the decomposition even at the beginning went as far as the production of the dissociable electrolytic molecules.

With the view of obtaining some preliminary notion of the magnitude of the changes which were to be expected, I made a series of experiments on the growth of ordinary putrefactive bacteria in blood and serum, and of pure cultures of certain bacteria in solutions of Witte's "peptone." The freezing-point determinations were made by means of a modification of Beckmann's apparatus, and the electrical resistance was measured by the telephone method of Kohlrausch. It

will be seen from the subjoined specimens of the results that very striking changes are observed both in the total molecular concentration and in the electrical conductivity, which, upon the whole, run a parallel course. Thus, in Experiment I, dog's defibrinated blood and serum were allowed to putrefy for 6 weeks in a bath at a temperature of 37° to 39° C. The electrical conductivity of the blood in that time was increased more than eleven-fold, and the freezing point was depressed in proportion. There was a similar change in the serum, although the maximum was much less. Curves plotted from the results of this experiment are shown in Plate V.

In Experiment II, three specimens of blood taken at the same time from the carotid artery of a dog were allowed to putrefy first at the air temperature, then at 35° C., and then at 40° to 43° C. Of course in all the experiments evaporation was prevented. The course of the decomposition is shown in the curves of Plate VI, which represent the electrical conductivity. It is abruptly hastened by raising the temperature to 35° C. Ultimately the conductivity reaches a maximum and then remains practically constant for an indefinite period. At the time when the maximum was reached the blood was seen to be crowded with bacteria which had formed spores. It is to be presumed that at this point the conditions had become unfavorable for the growth of the bacteria, owing to the increase of osmotic pressure due to the accumulation of the products of their activity or to some specific toxic action of these products and the using up of the nutritive material. A specimen of the blood at this stage was diluted with its own volume of water so as to diminish the concentration of the waste-products, and a renewed period of growth, indicated by a steadily mounting electrical conductivity, was again observed. It may seem strange that bacteria should be able to grow at all in a liquid whose osmotic pressure is 8 or 10 times as great as that of normal blood-serum or the cell-sap of ordinary vegetable cells. But Wladimiroff * has shown that the osmotic pressure in the interior of bacteria is extraordinarily great, more than 3 times that of the cells of the higher animals. And if the bacterial cell-wall is freely permeable to any of

* *Zeitsch. f. physikalische Chemie*, vii, 529.

the decomposition products, as, for example, the red blood corpuscles are to urea, these products, although they would diminish the freezing point would not exert any osmotic pressure on the cell-wall.

Experiments III and IV show the result of observations on the growth of B. subtilis, B. proteus Zenkeri, B. proteus vulgaris, B. Friedländer, B. coli communis, Bacillus of hog-cholera, and B. lactis aërogenes. The cultures were all grown in a solution of Witte's peptone. Plates VII and VIII represent in graphic form the results of Experiment III.

As to the nature of the electrolytes produced by the bacteria, we know already numerous bodies, especially among the decomposition products of putrefying proteids, which are capable of acting as ions, for example, such organic kations as the amines and ammonium, and such anions as the acids of the fatty series.

I shall not further discuss these results at present. They are enough to show that in such observations we have a practical method of measuring the amount of decomposition produced by, and therefore the rate of growth of, bacteria. It is possible that sufficiently great and constant differences might be observed between different kinds of bacteria when grown in the same or in different liquid media to enable us to use the method as a supplement to our present means of diagnosing between nearly related forms. And for this purpose, since the curves of concentration and conductivity run practically parallel to each other, it may be that measurements of the electrical conductivity alone might suffice. These would have the great advantage over measurements of the freezing point that they could be carried out in tubes furnished with platinum electrodes fused through the glass, or perhaps passing through the plug, and the necessity of opening the tubes would thus be obviated.

It is hoped that further observations, now in progress, may cast light upon this and some of the other interesting questions arising out of this research.

Tabulated Results.

The conductivity (λ) is given in reciprocal ohms $\times 10^8$ and reduced to 5° C. In the column headed Δ the lowering of the freezing point is given in degrees centigrade.

EXPERIMENT I.—March 30, 1898. PUTREFACTION.

		Δ.	λ (5°)×10⁸.				Δ.	λ (5°)×10⁸.	
Mar. 30.	Dog's defibrinated blood (fr.). (Sp. gr. 1057.0; ash 1.544%.)	.628	33.80	Air temperature.	Mar. 30.	Serum from clot of same blood. (Sp. gr. 1022.3; ash 0.925%.)	.628	82.44	
Apr. 2.			34.16	" "	Apr. 2.				Put in bath at 37°–39° C.
" 6.			31.41	" "	" 5.		.759		
" 11.			28.35	" "	" 6.		.834	97.99	
Mar. 30.	Same dog's defib. blood (fresh).	.628	33.80	" "	" 8.		.832	104.24	
Apr. 2.			34.16	" "	" 11.		1.308	150.63	
" 5.		.632		Now put in bath at 37°–39° C.	" 16.		2.229	220.43	
" 6.			55.47		" 25.		2.652	232.57	
" 8.		1.271	81.77	At 2.30 p. m.	May 15.		2.814	277.05	
" 8.			83.25	At 5.25 p. m.					
" 11.		2.043	130.00						
" 13.		2.784	154.10						
" 16.		3.506	202.97						
" 25.		7.417	337.10						
" 27.			342.57						
May 15.		8.1 (?)	371.15						
Apr. 25.	The defibrinated blood which had been kept in bath + 1 volume water.	3.072	232.57						
May 15.	" "	4.234	335.76	Kept in bath since Apr. 25.					
" 15.	The defibrinated blood which had been kept in bath + 3 volumes water.	1.974	218.53						

EXPERIMENT II.

Jan. 4, 1898.

PUTREFACTION. DOG'S DEFIBRINATED BLOOD. THREE SAMPLES, A, B AND C, FROM THE SAME DOG. A AND B IN STERILE FLASKS; C, NOT.

	$\lambda_{(5°)} \times 10^8$. A.	$\lambda_{(5°)} \times 10^8$. B.	$\lambda_{(5°)} \times 10^8$. C.	
Jan. 4.			42.82	
" 9.	56.58		37.61	A was opened for the first time to-day, and is laked.
" 11.	60.21		45.02	A and C are both completely laked.
" 13.	65.67	63.66	48.32	B was opened for the first time to-day, and is completely laked.
" 15.	75.44	66.09	58.01	
" 20.	90.21	79.96	68.88	
" 28.	101.81	93.12	77.28	
Feb. 1.	126.75			A was put in bath at 30°–36° C. 30 hours before this measurement.
" 4.	128.68	99.41	82.71	A, B and C were put in bath at 35° C. to-day.
" 5.	139.64		90.37	
" 7.	183.70	143.47	102.80	Temperature of bath now raised to 40°–43° C.
" 8.	232.57	162.50	115.75	
" 9.	274.05	178.52	124.45	
" 10.	308.02	214.47	147.65	
" 16.	359.58	314.13	232.57	
" 24.	356.04			
" 25.	359.58	381.78	318.87	
" 26.	363.70	390.00		
" 28.	364.23	393.02	338.00	
Mar. 2.	360.09	397.96	343.03	
" 3.		403.02	345.37	
" 7.	359.07	397.96	347.26	
" 11.	349.65			
" 22.		399.85	340.27	
Apr. 27.	382.93	396.09	343.03	

From Jan. 4 to Jan. 31, A, B and C were all kept at room temperature. When A and B were first opened (on Jan. 9 and 13, respectively) neither was found completely sterile. A rod-shaped bacterium was present in both, but there was no putrid odor, although this was very marked in C. After being opened A and B were thoroughly exposed to the air and allowed to putrefy like C.

EXPERIMENT III.

A STERILE SOLUTION OF WITTE'S PEPTONE WAS PREPARED. ON APRIL 11, 1898, 6 TUBES WERE INOCULATED WITH BACILLUS SUBTILIS AND 4 TUBES WITH BACILLUS PROTEUS ZENKERI.

		Δ.	$\lambda_{(5^\circ)} \times 10^8$.	
1898. Apr. 5.	Sterile peptone solution.	.124	13.68	Ash .0876 grm. / Solids 4.3052 grm. } in 100 cc.
" 11.	Sterile peptone solution (after further sterilization in steam sterilizer.)	.101	11.25	
" 11. 1 p. m.	Inoculated 6 test-tubes with B. subtilis and 4 with B. proteus Zenkeri.			
" 13. 4 p. m.	B. subtilis No. 1.	.104	12.12	At 35° C. since inoculation. Considerable growth.
	B. proteus Zenkeri No. 1.	.114	11.53	At 35° C. Less growth.
" 16.	B. subtilis No. 2.	.121	13.65	At 35° C. for 3 days; then room temperature since.
	B. proteus Zenkeri No. 4.	.104	12.27	" "
" 27.	B. subtilis No. 3.	.152	16.42	In bath at 40° C. since April 20.
May 15.	B. subtilis No. 4.	.571	51.57	In bath at 40° C. since April 27.
" 15.	B. proteus Zenkeri No. 3.	.134	13.37	At room temperature.
" 15.	B. proteus Zenkeri No. 2.	.125	12.56	At room temperature. Very little growth.
" 15.	B. subtilis No. 5.	.231	21.91	At room temperature since inoculation. Fair growth.
" 15.	B. subtilis No. 6.	.219	19.03	At room temperature since inoculation. Fair growth.
Apr. 13.	B. subtilis No. 1.	.104	12.12	Now exposed to air and put in bath at 40° C.
" 16.	B. subtilis No. 1.	.589	65.57	Far more growth than in unopened tubes.
" 25.	B. subtilis No. 1.	1.267	144.45	
May 15.	B. subtilis No. 1.	1.482	171.05	
" 15.	B. subtilis No. 3.	1.197	142.98	Exposed to air on April 27. In bath at 40° C. since.
Apr. 13	B. proteus Zenkeri No. 1.	.114	11.53	Exposed to air after opening and put in bath at 40° C.
" 16.	B. proteus Zenkeri No. 1.	.530	59.97	
" 25.	B. proteus Zenkeri No. 1.	1.093	132.86	

EXPERIMENT IV.—April 18, 1898, 1 P. M.

TWO TUBES OF THE SAME SOLUTION OF WITTE'S PEPTONE WERE INOCULATED WITH CULTURES OF THE FOLLOWING BACTERIA. ALL WERE PUT IN THERMOSTAT AT 35° C., EXCEPT B. SUBTILIS AND B. PROTEUS VULGARIS, WHICH WERE LEFT AT ROOM TEMPERATURE (10°–15° C.). BUT ON APRIL 27 B. SUBTILIS WAS PUT IN BATH AT 40° AND KEPT THERE TILL MAY 15.

	B. subtilis.		B. proteus vulgaris.		B. Friedlander.		B. coli communis.		B. of hog-cholera.		B. lactis aërogenes.	
	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.	Δ.	$\lambda_{(5°)} \times 10^8$.
Apr. 22, 2.35 p.m.					.154	17.95	.181	16.98	.142	17.40	.181	20.76
Apr. 27, 11.50 a.m.			.156	20.15								
May 12.					.207	20.05	.187	19.69	.167	16.52	.246	25.59
May 15.	.318	36.31	1.343*	158.52								

Control peptone tube in thermostat till May 12, when $\Delta = .148$ and $\lambda_{(5°)} \times 10^8 = 16.10$.

* Test-tube opened on April 27 and left in bath at 40° C. with ordinary cork till May 15.

DESCRIPTION OF PLATES V-VIII.

PLATE V.

Curves plotted from the results of Experiment I to show the changes in the molecular concentration of putrefying blood and serum.

FB, freezing point of blood; *FS* of serum; *CB*, conductivity of blood; *CS* of serum. *A* shows the course of the curve of conductivity in the defibrinated blood kept at air temperature. On April 2 the blood and serum were put in a bath, the temperature of which varied between 37° and 39°.

PLATE VI.

Curves plotted from the results of Experiment II to show the changes in the electrical conductivity of putrefying blood.

A, *B*, *C*, curves of three samples of blood taken at the same time from the carotid of a dog.

a shows the point at which (on Jan. 28) *A* was put into bath at 30°-36° *C*; *b*, the point at which (on Feb. 1) it was taken out of the bath. The dotted line passing through the three curves and labelled "Bath" shows the point where (on Feb. 4) *A*, *B* and *C* were put into bath at 35°, where they were afterwards kept. *I* is a curve from a specimen of blood from another dog, the numerical results of which are not quoted.

PLATE VII.

Shows the changes in the conductivity of the contents of five tubes (Nos. 1, 3, 4, 5 and 6) inoculated with Bacillus subtilis on April 11. The curves are plotted from the results of Experiment III.

PLATE VIII.

Shows the changes in the freezing point of the contents of the same tubes.

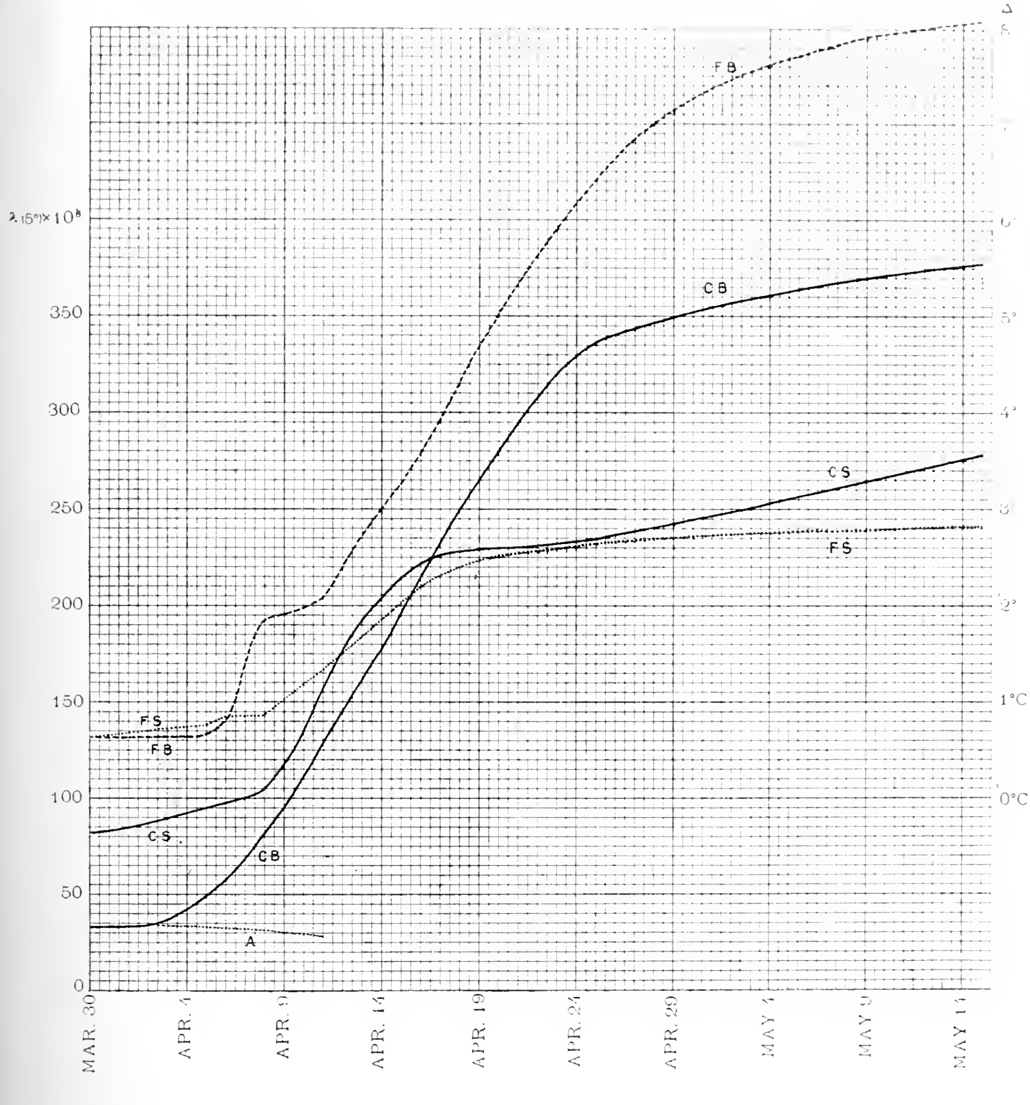
On April 13, No. 1 was freely exposed to the air and then put in a bath at 40°, corked tightly with an ordinary cork.

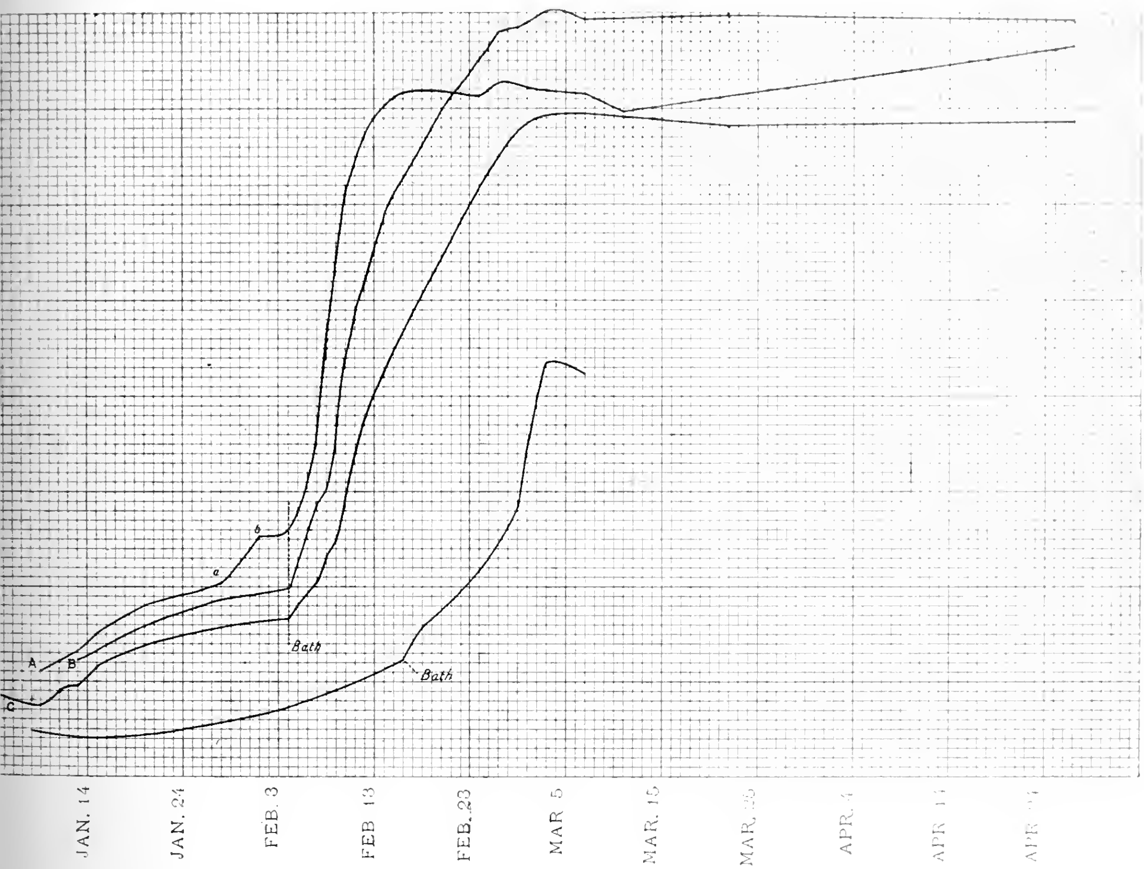
On April 27, No. 3 was treated in the same way.

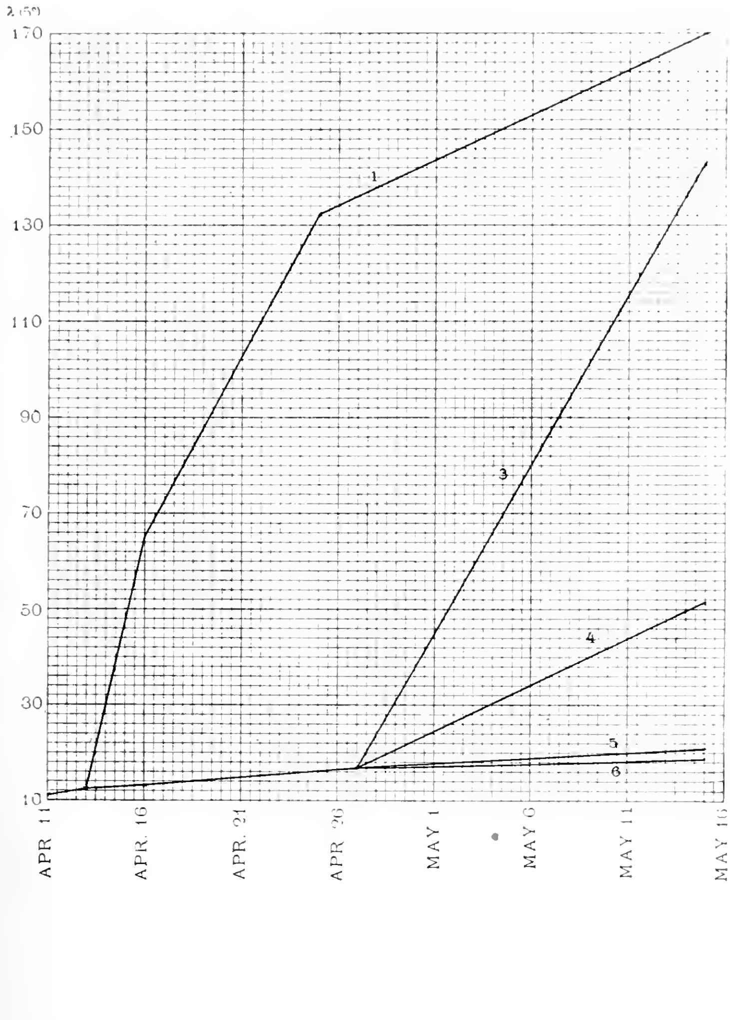
No. 4 was put in the bath at 40° on April 27 and kept there till May 15, when it was opened.

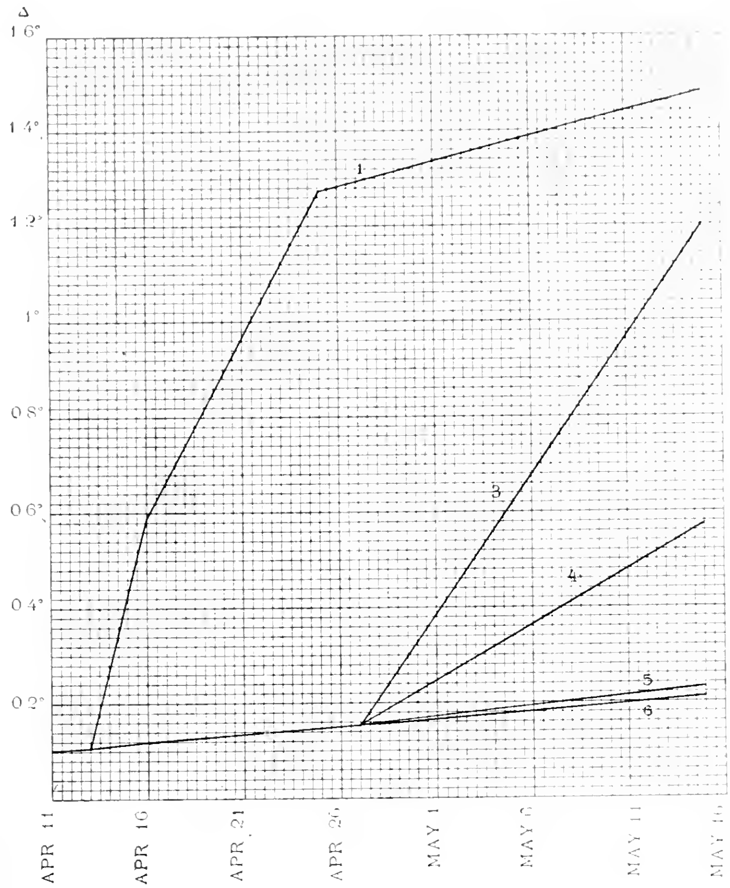
Nos. 5 and 6 were kept at the room temperature.

The curves of conductivity (Plate VII) and molecular concentration, it will be seen, run nearly parallel to each other.


λ (5°)×10⁸
350
300
250
200
150
100
50
0
FB
CB
CS
FS
A
1°C
0°C
MAR. 30
APR. 4
APR. 9
APR. 14
APR. 19
APR. 24
APR. 29
MAY 4
MAY 9
MAY 14


A
B
C
a
b
Bath
Bath
JAN. 14
JAN. 24
FEB. 3
FEB 13
FEB. 23
MAR 5
MAR. 15


170
150
130
110
90
70
50
30
10
1
3
4
5
6
APR 11
APR. 16
APR. 21
APR 26
MAY 1
MAY 6
MAY 11
MAY 16


Δ
1.6°
1.4°
1.2°
1°
0.8°
0.6°
0.4°
0.2°
1
3
4
5
6
APR 11
APR 16
APR 21
APR 26
MAY 1

ON SUPRA-ARTERIAL EPICARDIAL FIBROID NODULES.

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(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

PLATES IX–XI.

The morbid condition to be described in this article is characterized by the presence of multiple, grayish-white, fibroid nodules situated upon the coronary arteries of the heart. Although similar nodules have doubtless been previously observed, I have been unable to find any satisfactory description of them in medical literature. I have, therefore, taken advantage of the opportunity to study histologically five well-marked examples which, during the last few months, have been observed among the autopsies at the Johns Hopkins Hospital.

These supra-arterial nodules differ from the ordinary tendinous or milky patches, often observed upon the epicardium, especially over the right ventricle, in their multiplicity, their smaller size, and their distribution over the coronary arteries to which they evidently bear some definite relationship. In gross appearance they resemble much more closely the nodular affection of the arteries first described by Kussmaul and Maier* in 1866 under the name "periarteritis nodosa." In consequence of this resemblance, which, however, is only superficial and does not pertain to the histological structure, it is appropriate to direct attention to the latter affection.

Kussmaul and Maier observed nodular thickenings on all the smaller arteries of the body, except those of the central nervous system, in a man aged 27 who, after leading a vagabond's life, entered the hospital at Freiburg complaining of weakness and muscular pains. He grew gradually worse, had an intermittent temperature, suffered from gastro-intestinal disturbances and, late in his illness, with a progressive paralysis

* *Deutsches Arch. f. klin. Med.*, 1866, i, 484.

which showed from time to time short remissions. There were also symptoms of nephritis. He was ill four weeks.

At autopsy the most noticeable feature was the presence of countless opaque nodes on the smaller arteries of the body. The pericardium was smooth except where the coronary arteries were studded with these uniform grayish-white patches.

Microscopical examination showed usually no changes in the intima of the arteries, but there was a cellular infiltration and degeneration of the media, with infiltration and much hypertrophy of the adventitial coat, the lumen of the vessel being usually diminished, but occasionally dilated. Kussmaul and Maier expressed the opinion that the arterial changes were primary, and that the condition was a hitherto undescribed affection characterized by well-marked symptoms. They had no suggestion to make as to the cause of the arterial changes.

Chvostek and Weichselbaum * reported in 1877 the case of a soldier, aged 23, who became suddenly ill, complaining of headache, dizziness and nausea. There followed irregularity of the pupils and paralysis of the left ocular nerve, of the right side of the face and of the right extremities. He died after an illness of four months.

On section a ruptured aneurism of the left arteria profunda cerebri (posterior cerebral) was found, with hæmorrhage into the cortex. Many of the medium-sized and small arteries of the body, but only at their bifurcation, were the seat of opaque gray swellings varying in size and shape. There were caseous areas in the lungs, pleuræ, liver and kidneys.

Microscopical examination of some of the arterial tumefactions showed an increase in the cells of the intima with small-celled infiltration. These changes extended through the media and met a like proliferation of the adventitial coat, the elastic tissue offering the most resistance. There were aneurismal dilatations of some of the affected arteries. The condition was primarily, they consider, an endarteritis of luetic origin resembling that described by Heubner.† There were no other suspicious lesions nor was there a syphilitic history.

A somewhat similar case is described by P. Meyer.‡ A soldier, 24 years of age, after a life of dissipation, was admitted to a hospital complaining of great muscular pain, headache, sleeplessness, irregular fever and weakness. These symptoms became more marked and death took place in two months.

* *Allg. Wiener med. Zeitung*, 1877, xxii, 257 *et seq.*

† Die leutische Erkrankung der Hirnarterien, etc., Leipzig, 1874.

‡ Virchow's *Archiv*, 1878, lxxiv, 277.

The smaller arteries of the body were found at autopsy to be the seat of several yellowish-white dilatations from a mustard seed to a pea in size, most frequently situated at the branching of the artery involved. The vessels of the brain and cord were quite normal. The primary change Meyer conceives to consist in the rupture of the elastic coat of the artery leading to a dilatation of the other layers with thrombus formation and subsequent proliferation of the arterial walls.

He thinks that sudden changes in blood pressure from the ingestion of large quantities of fluid, from great exertion and from dissipation had weakened and broken through the strong elastic fibres with the above-mentioned secondary results.

This view is supported in part by Eppinger,* who brings the affection into line with aneurisms produced by increased blood pressure acting upon arteries whose elastic tissue is in places congenitally weak, and producing a bowing out of the wall with secondary hypertrophy of the intimal and adventitial layers.

The cases thus far have been those of young men, but that the process is not limited to this class is shown by the report of Fletcher.† A widow, aged 49, presented herself at the hospital complaining of muscular pain and weakness with occasional swelling of the legs. Later there were also intermittent fever, cough and albuminuria. The diagnosis rested between typhoid fever and miliary tuberculosis. Death occurred after two months.

The anatomical findings were very similar to those of von Kahlden,‡ who, in 1894, reported two cases in which like alterations in the arteries were observed. Both patients were women beyond middle life, 49 and 52 years of age, the latter the mother of five normal children. In each case the symptoms were increasing weakness and fever to which in the older woman were added great pain and gastro-intestinal disturbance. The duration of the illness was 8 and 12 weeks respectively. On section, each of these three cases showed upon the small and medium-sized arteries circumscribed whitish nodules, most abundant in the heart, pericardium, mesentery, pleuræ, liver and kidneys, the arteries of the central nervous system being unaffected.

Fletcher and von Kahlden agree with Chvostek and Weichselbaum in considering that the process begins by a proliferation of the intima followed shortly afterwards by a like process with leucocytic infiltration in

* Pathogenesis, Histogenesis u. Aetiologie der Aneurysmen, Berlin, 1887.
† Zeigler's *Beiträge*, 1891, xi, 323.
‡ Zeigler's *Beiträge*, 1894, xv, 587.

the adventitial layer. This cellular tissue, they assert, invades the media on both sides leading to its rupture and to the degeneration of both muscular and elastic tissues, with an accumulation of small round cells and fibrin: a thrombus filling the lumen in the later stages completes the picture. They do not accept the hypothesis that the affection is due to syphilis on account of the complete absence of luetic history and lesions, but they are disposed to consider the causal agent to be some other, bacterial or toxic, infection of the blood which attacks the internal coat of the vessels and produces those changes which they consider to be primary.

From this brief review there would appear to be several reported instances of an affection of the smaller arteries, characterized macroscopically by circumscribed whitish nodules distributed pretty generally through the body, except in the central nervous system, and microscopically by hypertrophy of both internal (except in the case of Kussmaul and Maier) and adventitial coats with weakening, and, in places, with rupture of the elastic coat. There was sometimes dilatation of the vessel, but usually the lumen was narrowed.

The lesions in these cases were accompanied by clinical symptoms having much in common. The onset was sudden and marked by muscular pain, weakness, intermittent fever and gastro-intestinal disturbances. There were occasionally also subsequent paralysis, anæmia and nephritis. The course of the disease was progressively fatal, death resulting in all cases in from 7 to 12 weeks.

Three suggestions have been made as to the etiology. Chvostek and Weichselbaum reasoning from the close resemblance to arterial changes of known syphilitic origin, consider lues to be the cause of the lesions. Meyer and Eppinger think that the primary change is a weakening of the elastic membrane; while Fletcher and von Kahlden are of the opinion that the proliferation of the intima is the first step, and that this is brought about by the direct action of bacteria or of toxins.

The gross appearance of the nodules on the surface of the hearts now under consideration agrees closely with that of the arterial thickenings scattered generally through the body in the cases of so-called periarteritis nodosa.

Description of gross specimens (Plate XI). In the specimens examined the extent of the process varied much, from tortuous, more or less uniform, elevations over the arteries, to whitish dots minute in size and few in number which almost escape attention. In most of the specimens the epicardium between the arteries was delicate, the only superficial lesion, other than an occasional " milky patch," being the grayish-white nodules along the smaller arteries. These elevations may be separated from each other by wide intervals or they may be quite close, resembling beads strung along the vessel, or they may coalesce throughout the entire length of the artery (Case III).* They hold no demonstrable relationship to the points of bifurcation of the vessels, nor was any particular branch especially affected. In no case did the thickening tend to encircle the artery. In one case (Case V) they were noticed, not only over the ventricle, but also, although few in number, upon the auricle, and over the small vessels in the adventitia of the aorta in its ascending part, where a single small focus measuring 1x2 mm. in diameter existed. In the same case a few scattered nodules were present also over the large veins (Plate XI). There was no tendency to aneurismal formation.

Microscopical.—The appearances observed under the microscope differ somewhat in different cases, although the main lesion is, as I hope to show, constant. In Cases I, II, III and IV the following conditions were observed in cross-sections including the coronary arteries with the nodules upon their adventitial surfaces. The intima of the arteries in the majority of sections examined showed little alteration; there may be slight swelling and the inner margin may show a somewhat irregular surface, which is not excluded from being the result of irregular contractions of this coat in the hardening process. In several specimens there could be made out a marked increase in the intimal layer, either as a single mass bulging into the lumen, representing perhaps an organized mural thrombus or as several smaller, more diffuse protrusions consisting of fusiform and branched cells with some intercellular substance. In the last case examined (Case V) the intimal changes are much more pronounced, being most marked in situations corresponding to the nodes. The entire internal coat is thickened, but the increase is especially marked in the myocardial hemispherical segment of the artery. In this situation it is fully six times its normal thickness and the muscular tunic is here somewhat diminished and degenerated. The increase in the thickness of the intima on the epicardial side is relatively slight, although the muscle in this region is degenerated and attenuated to a greater degree than on the opposite side of the vessel.

* Protocols of the cases are appended to this article, pp. 257–260.

The muscular coat (media) is in the main, except in the instance to be presently mentioned, unchanged, so far as can be determined, in the sections stained by hæmatoxylin and eosin. Occasionally there is an apparent degeneration of muscular fibres as shown in a reduction in number of muscle-cell nuclei and there is exceptionally an infiltration with small round cells. In Case V, as already mentioned, the media shows much more pronounced pathological changes, in that this tunic is reduced in thickness and areas of hyaline metamorphosis are apparent.

The changes in the adventitia proper are slight and inconstant. Only occasionally is there any hypertrophy.

The nodular formations lie upon the vessels within the epicardium, being seated primarily in the layer of connective tissue between the endothelial covering and the delicate layer of elastic fibres which rests upon the main layer of loose vascular connective tissue containing the epicardial fat. Their situation corresponds, therefore, to that of milky patches as determined by Ribbert.* In their immediate neighborhood are found the usual loose adipose and connective tissues, vessels and nerves. But the nodules differ from the normal connective tissue, being at once distinguished from this through their dense and fibrous, often sclerotic, appearance.

The appearance on cross-section is as though a compact mass of firm connective tissue, convex on its inner surface, were set upon the artery in the loose epicardial tissue. The lateral edges of the thickening slant gradually upward to the surface and are continuous there with the epicardium (Plates IX and X). Scattered through the firm mass are a few fusiform connective-tissue cells. At the base of the area and at the sides are often groups of lymphocytes and sometimes a small number of polymorphonuclear leucocytes (Plate IX, Fig. 1 and Plate X, Fig. 1). The firm tissue pushes up beyond the level of the rest of the epicardium and forms the opaque nodule seen in the gross specimen. The endothelium and occasionally some subjacent tissue cover the prominence and are continuous with the serosa over the rest of the heart. Beneath the nodules the layer of epicardial elastic tissue can usually be demonstrated, but the subjacent loose connective and adipose tissues are more or less atrophied.

The size of the fibrous thickening varies within wide limits, both in depth and in lateral extension. It may form a comparatively narrow band which is not raised above the surface of the epicardium; or, again, it may appear as a high, irregular, almost pediculated projection rising from a compact base above the vessel (Plate X, Fig. 2).

* Virchow's *Archiv*, 1897, cxlvii, 211.

The nodule may not only cover the outer surface of the vessel but may extend a considerable distance in the loose cellular tissue on each side (Plate X); or, on the other hand, there may be an oval patch over only a portion of an artery, the remaining peri-adventitial covering being quite normal (Plate IX, Fig. 1).

The early stages of the process leading to the supra-arterial nodules presented a tissue richer in cells, both fibroblasts and lymphoid cells, situated superficially to the vessel and on its epicardial side. As the nodule becomes older, the more homogeneous and less cellular becomes its structure; the nuclei are fewer and the focus is more sharply differentiated from the surrounding tissue. In no instance was any tendency to a similar fibrous formation noticed on the side of the affected vessel next to the heart muscle, nor were any similar alterations seen about the arteries in the substance of the myocardium.

We have, then, in these cases, nodules on the surface of the heart macroscopically resembling those described by Kussmaul and Maier but totally dissimilar in their minute structure and relations; for the specimens stained in hæmatoxylin and eosin failed completely to show any constant degeneration or proliferation of the arterial walls, such as is present in periarteritis nodosa. The uniform relationship to arteries, however, suggested that there was probably some alteration in the vessel which was at least associated with the nodule. Hence a representative number of the sections were stained for elastic tissue by the fuchsin method of Manchot * and by the method of Weigert†.

Results of stain for elastic tissue. There were of course variations in the sections, but the general results were sufficiently uniform to be quite suggestive. The stains brought out the yellow elastic fibres in dark-red or violet, in contrast to the pale, partly decolorized tissues. In a few of the sections there were distinct breaks in the inner elastic coat just opposite the nodule.

The most noticeable alteration, however, and one present in two-thirds of the specimens was a diminution in the strength of the *outer* elastic coat between the muscle and adventitial layers. This membrane was well represented in the inner (myocardial) side of the artery, often by a heavy dark band, but toward the outer (epicardial) half it became

* Virchow's *Archiv*, 1890, cxxi, 111.

† *Centralbl. f. allg. Path.*, 1898, ix, 289.

thinned, the fibres appeared looser and separated and more or less interlaced by the coarser bands of the adventitial coat until, beneath the thickening over the vessel, perhaps the last trace of the elastic membrane disappeared (Plate IX, Fig. 2).

In no instance was this change accompanied by an increase in the thickness of the inner elastic layer; in fact, at times the latter also appeared reduced.

The extent of this reduction or disappearance of the outer elastic coat differed widely. The thinning of the elastica was often present not only beneath the fibroid nodule, but extended a considerable distance in each direction, affecting perhaps half the circumference; or, again, it was more localized and perhaps somewhat to one side of the thickest part of the node (Plate X, Fig. 2).

The normal relative strength of the inner and outer elastic layers varies considerably; usually the inner coat is the stronger and the fibres seem more compact, but in other specimens it appears to be the weaker of the two and the main elasticity of the vessel wall is evidently supplied by an outer membrane which is thick and firm. In a rupture of this strong band there is usually not a simple break in the continuity of its fibres, but they split up into a perfect meshwork, spreading through the adventitia (Plate X, Fig. 2). Elastic fibres were made out in some instances in the nodule, more commonly as a definite layer between it and the underlying connective tissue (Plate IX, Fig. 2). Occasionally there was a proliferation of elastic fibres through the thickened intima. Only rarely were defects in either elastic coat seen in the inner (myocardial) side of an artery. In these cases no alteration corresponding to the nodule under discussion was noticed in the remaining layers nor in the surrounding tissue. No intrinsic changes in the elastic fibres suggesting degeneration were made out; the only microscopical deviation from the normal was the apparent mechanical separation and the disappearance of the fibres. Several sections containing arteries not surmounted by nodules were stained in the same manner, and although irregularities in the elastic coats were found, there was not in any case the marked alteration described in the elastic membrane as existing beneath the fibrous thickenings.

Sections of the diffuse areas of epicardial thickening, known as "milky patches," showed the appearances described by Ribbert.* No relationship of these to blood-vessels was observed.

* Virchow's *Archiv*, 1897, cxlvii, 207.

The foregoing microscopical observations indicate a relationship between the fibroid nodules and the demonstrated weakening in that part of the arterial wall immediately beneath the nodule.

In considering the etiology of this certainly distinct lesion, one must recall the diseases with which it may be associated.

(1) There was a distinct history of syphilis in one case (III); in three it was denied (I, II and V), while there is reason to be quite sure that there was no luetic infection in the fifth case (IV). The nature of the histological changes does not correspond to that of a syphilitic affection. The arterial alterations produced by lues consist in an endarteritis associated usually with a periarteritis, or the direct inclusion of the vessel in surrounding gummatous material. In the sections examined the arterial walls were unaffected, except in the manner already described, and in no instance were caseous areas seen in the adjacent tissues.

(2) As far as the gross appearances go, the nodules might possibly be mistaken for tubercles. One of the cases reported had suffered from a general tuberculous peritonitis (Case III) for which laparotomy had been performed. In the other cases there was no evidence whatever, clinical or anatomical, of infection with the tubercle bacillus nor was there the slightest sign of any tuberculous structure in any of the preparations.

(3) It will be recalled that blood infection from either bacteria or their toxins was the cause suggested for periarteritis nodosa by the later writers, Fletcher and von Kahlden. They based their hypothesis upon the primary proliferation of the intima which they attributed to the direct action of toxic substances in the circulation. This cellular increase in the intima, as has been shown, is conspicuous chiefly by its absence in most of the sections from our cases, although it may be present, as in Case V. Moreover, cultures from the heart's blood were negative in two instances, while Staphylococcus pyogenes albus alone was found once, as was also in one case Staphylococcus pyogenes aureus. In the fifth case blood cultures taken during life remained sterile. It would be quite impossible to imagine that the nodules could be due to poisonous agents carried in the blood stream

and producing this fibrous thickening on but one side of the artery, while they so rarely affect a vein and do not injure further the intermediate arterial coats.

We may, therefore, doubtless exclude syphilis, tuberculosis and other infections as essential etiological factors in the production of these supra-arterial nodules.

The evidence seems to me strongly to support the view that the primary, underlying cause is to be found in a weakening of the arterial wall, due usually to defects in one or both of the principal elastic lamellæ of the artery, most frequently of the elastica externa. A number of facts in the protocols lend color to this theory. All the cases were in males between 19 and 54 years of age, accustomed, with one exception (Case III), to hard work, irregular methods of life, indifferent nourishment and varying quantities of alcoholic beverages. The heart was hypertrophied in four cases (I, II, IV, V); in one (IV) there were valvular lesions, in two (II and V) arteriosclerosis, in four (I, II, III, V) nephritis, in four (II, III, IV, V) œdema, and in a single case (I) aneurisms existed. These conditions indicate that during life there must have been irregularities in the force of the blood pressure, influenced further, doubtless, by the ingestion of large quantities of fluid.

The duration of the final illness varied from 8 weeks to 2 years. So far as known, no symptoms are attributable to the epicardial nodules.

As a probable explanation, then, of the origin of these fibroid nodules it is suggested that there is a weakening of the arterial coats, mainly of the outer elastic coat, on the side toward the nodule. While this defect may be congenital, it is more probable that it is acquired through poor nutrition combined with sudden alterations in blood pressure from the causes already indicated. This loss of elasticity, it may be supposed, is compensated by a fibroid thickening, situated not in the vessel wall, but beyond it in the epicardial tissue.

For such an explanation an analogy is to be found in the views now generally held of the formation of aneurisms, first advanced by Rokitansky and later supported, with certain minor modifications, by

Eppinger, P. Meyer, Manchot, Thoma and others. These authorities consider the primary change to be a giving away of the media, especially of the elastic laminæ and fibres, with subsequent bulging out of the arterial wall. When the weakening takes place slowly, cellular proliferation occurs in the intima and adventitial coats and the danger of aneurism is lessened by the thickening of the arterial wall. Tears in the elastic lamellæ are not uncommon, and may occur without the formation of aneurism. The high pressure in the coronary arteries would seem to render these vessels particularly exposed to such injuries, when the nutrition of their walls is impaired. Of especial interest in our cases is the demonstration of more frequent and pronounced defects in the outer elastic lamella than in the internal one.

It will be recalled that in no case was there an increase in the surrounding connective tissue corresponding to the inner or myocardial side of the artery and it is to be conjectured that here the heart muscle affords sufficient support to prevent stretching of the vessel walls with such ruptures of the elastic lamellæ as were observed on this side. Each pulsation must produce expansion of the artery, which is most marked at the point of least resistance, *i. e.* at the outer or epicardial surface. Excessive or irregular expansion can easily be thought of as injuring more or less the elastic coat. In such cases there follows a still greater protrusion outwards of the vessel wall with each pulsation. The significant localization of the defects in the outer elastic lamellæ upon the epicardial side of the arteries indicates the greater exposure of the artery upon this side to injury, and this may be due to the lack of the support which is afforded to the myocardial side of the artery by the surrounding tissues. The firm resistance offered to the expansion of the vessel on the inner side by the ventricular wall is an important factor in favoring increased bulging in the free semi-circumference. The absence of this counter-support probably accounts for the fact that similar nodules are rare upon the auricles.

SUMMARY AND CONCLUSIONS.

1. Fibroid nodules seated in the epicardium directly over branches of the coronary arteries of the heart are not uncommon. They may be present in large numbers and are found most frequently upon the

surface of the ventricles, but may occur over the auricles and even on the outer surface of the ascending aorta. They are rarely observed over the coronary veins.

2. While often resembling in gross and superficial appearances the nodules described by various writers under the name of "periarteritis nodosa," they differ from these in essential respects. They are seated outside of the adventitial coat and lie within the epicardium. They are composed of dense, fibrous, sclerotic tissue, poor in cells. In earlier stages of their formation they are richer in cells, both fibroblasts and lymphoid cells.

3. These supra-arterial nodules bear no definite relation to endarteritis, although they may be associated with this condition.

4. There were found with great regularity in the arterial wall immediately beneath the nodule, changes, which indicated a weakening of the wall in this situation. In some instances the muscular coat was thinned and degenerated, but the most common and important change was reduction and often disappearance of the elastic lamellæ and fibres, the outer elastic lamella being the one most frequently and intensely affected. These lesions were often limited to the segment of the arterial wall adjacent to the epicardium, the inner or myocardial segment of the same artery being free from similar alterations, or presenting them only in a slight degree. It is suggested that the absence on the outer or epicardial segment of the firm support afforded to the artery on the inner or myocardial aspect by the surrounding tissues renders the former more liable to damage to the elastic tissue resulting from irregularities and increase of blood pressure associated perhaps with defects of nutrition.

5. In consequence of the weakening in the arterial wall the artery would tend to bulge at the affected spot toward the epicardium were this tendency not restrained. The formation of the dense supra-arterial nodule of fibrous tissue over the weakened area holds this tendency in check and may therefore be regarded as an adaptive or compensatory change.

The question as to the immediate exciting cause of the new growth of tissue offers the same difficulties as that pertaining in general to

similar growths of connective tissue. Some would doubtless attribute it to direct stimulation from the pressure and shock of the impinging artery, others to defects in the tissue, and still others to a disturbance of the neighborhood relations of the part. It is not deemed necessary to enter into a discussion of these various hypotheses.

I take pleasure in expressing my thanks to Dr. Welch for examining my sections and to Dr. Flexner for constant advice and help.

PROTOCOLS OF THE CASES.

Case I. Man, G. J., aged 45, colored. Clinical history not obtainable.

Anatomical diagnosis. Enlargement of middle lobe of prostate gland; purulent cystitis, pyoureter, pyglonephritis, pyonephrosis.

Cardiac hypertrophy without valvular lesion: supra-arterial coronary nodules. Hæmorrhage from penis and stomach. Broncho-pneumonia. Emphysema. Exostoses on ribs.

Additional notes. Pericardium shows "milk patch" over right ventricle. The remainder is smooth except along the ramifications of the branches of the coronary arteries over both ventricles, where grayish-white, semi-translucent nodules, varying in size from an ultimate tubercle to one of a millimetre in diameter exist. These may be scattered at intervals along the vessel or in the case of the flatter and more opaque ones may almost coalesce throughout the entire length of the vessel. Apparently there is no bulging inward of the vessel wall corresponding to the thickenings. The valves are delicate. The left ventricle is thickened, not dilated. Weight of heart 370 grammes. No arterial nodules detected in any of the organs.

Bacteriological report. Heart and lungs, Staph. pyog. albus.

Case II. Man, S. C., aged 54, colored, coachman.

Clinical history. Patient came to hospital complaining of shortness of breath, cough and swelling of the legs and abdomen. Has led a rough, exposed life. Used tobacco and alcohol freely. Fifteen months before admission had attack of dyspnœa, which has been repeated many times since, together with œdema of face and of extremities and ascites. Much difficulty in micturition. Urine contains albumin and hyaline casts. Died eleven days after admission, in which time 3800 cc. of fluid were drawn from his abdomen.

Anatomical diagnosis. Arteriosclerosis. Aneurism of the thoracic and abdominal aorta. Cardiac hypertrophy. Supra-arterial coronary

nodules. Chronic passive congestion of the viscera. Chronic diffuse nephritis. Atrophy and cirrhosis of liver. Œdema and ascites. Ecchymoses in the serous membranes.

Additional notes. Pericardium generally smooth. Following the course of the coronary arteries and their branches are small grayish-white nodular thickenings from a pin's head to a millet-seed in thickness. Vegetations are present on the aortic segment of the mitral valve. A bulging is seen in the transverse arch of the aorta and a second dilatation in the abdominal aorta below the superior mesenteric artery.

Bacteriological report. Cultures from heart, lungs, peritoneal cavity, liver and spleen are negative.

Case III. Man, W. D., aged 43, colored.

Clinical history. Admitted to hospital complaining of swollen abdomen and dyspnœa. One brother died of phthisis. History of syphilis, gonorrhœa and abuse of alcohol. Patient felt well until 6 weeks before admission when he began to complain of headache, muscular pain, and weakness followed by cough, dyspnœa and ascites. He was transferred to the surgical side, laparotomy was performed and a general tuberculous peritonitis was found. Death occurred 18 days after operation, about 10 weeks after the onset of symptoms.

Anatomical diagnosis. Tuberculosis of peritoneum. Tubercles in form of large caseous and conglomerate masses. Purulent peritonitis. Operation wound for laparotomy. General tuberculosis of lymphatic glands and abdominal organs. Acute tuberculosis of left pleura. Hydrothorax. Double hydroureter and hydronephrosis. Interstitial orchitis.

Additional notes. Heart small. The coronary arteries generally are marked out by grayish-white elevations fusing together over them, forming tortuous opacities. The epicardium between these thickenings is normal except for some milky patches.. The intima appears smooth.

Bacteriological report. Cultures from lungs, heart, kidney and liver show Staphylococcus pyogenes aureus.

Case IV. Man, J. H., aged 19, white, florist.

Clinical history. Patient admitted to hospital complaining of cough, dyspnœa and palpitation. Three months previously had suffered with œdema of ankles, but resumed work until a month before admission, when the symptoms complained of made it necessary to stop. On admission there were marked evidences of aortic and mitral disease with loss of compensation and cardiac enlargement. He died at the end of three weeks after admission, during which time the symptoms had become worse.

Anatomical diagnosis. Proliferative endocarditis affecting aortic and mitral valves. Hypertrophy and dilatation of both sides of the heart. Relative insufficiency of mitral and tricuspid valves. Hypoplasia of aorta. Chronic passive congestion of lungs, liver, spleen and gastro-intestinal tract. Œdema, ascites, hydrothorax, jaundice.

Additional notes. Visceral pericardium smooth except for a few granules of fibrous thickening at the outer edge of auricle and over the right ventricle where there are a number of minute, rather translucent white granular spots, slightly elevated, lying over the course of vessels.

Posterior segment of chordæ tendineæ of mitral valve shortened and thickened. Aortic valve thickened along its free edge. No nodules in organs.

Bacteriological report. Cultures from peritoneal cavity, heart, spleen and mitral valve, negative.

Case V. J. R., man, aged 48, colored, upholsterer. Complained on admission of shortness of breath and swelling of legs. Had always done hard manual labor and used tobacco freely, otherwise fairly good personal history. Present illness began six weeks before admission, when shortness of breath upon exertion was first noticed. This became progressively more marked. Patient had some cough.

On admission there was present general œdema of lungs, abdomen, face and extremities with some cyanosis. Heart was enlarged but no organic lesion made out. Radial walls markedly thickened. Urine contained hyaline and granular casts and 0.2-0.7 per cent. albumin. Condition became gradually worse. Cheyne-Stokes breathing appeared and patient died seven weeks after admission, after illness of about three months.

Anatomical diagnosis. Arteriosclerosis; cardiac hypertrophy; thickening of mitral valve; extensive supra-arterial epicardial fibroid nodules; thrombi in auricular appendages; infarct of kidney; chronic nephritis; œdema and chronic passive congestion.

Additional notes. Heart weighed 540 grms. Hypertrophy, chiefly of left ventricle, with dilatation. Over the epicardial surface of the main coronary arteries of the left ventricle, as well as their branches and small twigs, are seated, discrete, small, elevated fibroid nodules of a semi-translucent or opaque appearance, resembling often beads strung along the course of the superficial vessels (Plate XI). Similar nodules are found in small numbers upon the auricles and upon the arch of the aorta. In these situations their relation to blood-vessels can also be demonstrated. Several similar nodules occur along the course of the larger branches of the coronary vein (Plate XI).

The coronary arteries are tortuous and extensively sclerosed. There is a general arteriosclerosis.

DESCRIPTION OF PLATES IX–XI.

Plate IX.

Fig. 1 (Case II).—Transverse section of small artery in epicardium, surmounted by a fibroid nodule. The fibrous thickening is over about one-half the width of the vessel. Hæmatoxylin and eosin staining. *A.* Intima. *B.* Inner elastic coat; wavy lines showing only at the sharper turns of the vessel wall. *C.* Media, slight swelling and degeneration (reduction in the number of nuclei) beneath the nodule. *I.* Adventitial coat unaltered. *F.* Normal epicardium. *G.* Supra-arterial fibroid nodule projecting above the surface of the epicardium and extending to the adventitial membrane. *J.* Accumulation of small round cells in the epicardium at the sides of the node. *K.* Nerves in cross-section. *L.* Capillaries. *M.* Portion of a vein evidently not associated with the fibrous nodule. *H.* Heart muscle.

Fig. 2 (Case II).—Specimen stained in fuchsin (Manchot's method). *A.* Intima. *B.* Inner elastic coat unaltered; no break or weakness beneath the nodule. *C.* Media. *D.* Outer elastic membrane between the muscle and the adventitial layer. This elastic membrane is thick and firm, and evidently supplying the chief support and giving the elasticity to the vessel wall. The membrane is intact, except at *E*, where beneath the nodule there is a marked fraying out and disappearance of the elastic fibres with a consequent diminution in the strength of the arterial wall. *F*, *G*, *H*, *I* as in Fig. 1.

Plate X.

Fig. 1 (Case II).—Same specimen as Plate IX, Fig. 2, stained by hæmatoxylin and eosin. *A.* Intima, with areas of proliferation probably not associated with the fibrous nodule. *B.* Inner elastic coat. *C.* Media apparently unchanged. *I.* Adventitial coat unaltered. *F.* Normal epicardium. *G.* Fibroid nodule projecting but slightly above the surface of the epicardium and extending to the adventitial coat beneath. *J.* Groups of small round cells at the side and beneath fibrous nodule. *K*, *L*, *M*, *H* as in Plate IX, Fig. 1.

Fig. 2 (Case III).—Transverse section of larger artery surmounted by a pronounced fibrous thickening, somewhat pedunculated and projecting abruptly from the surface. Same staining as Plate IX, Fig. 2. *A.* Intima. *B.* Inner elastic coat, very firm, unaltered. *C.* Media. *D.* Outer elastic coat, compact only for a small portion of the circumference on the myocardial side. From this point on each side the fibres may be seen to separate into a network and to become diminished in number until at *I* they cannot be demonstrated.

Plate XI.

Heart of Case V.—The supra-arterial epicardial nodules are well shown over the coronary arteries and their branches. To the left are seen a few nodules upon a large vein.

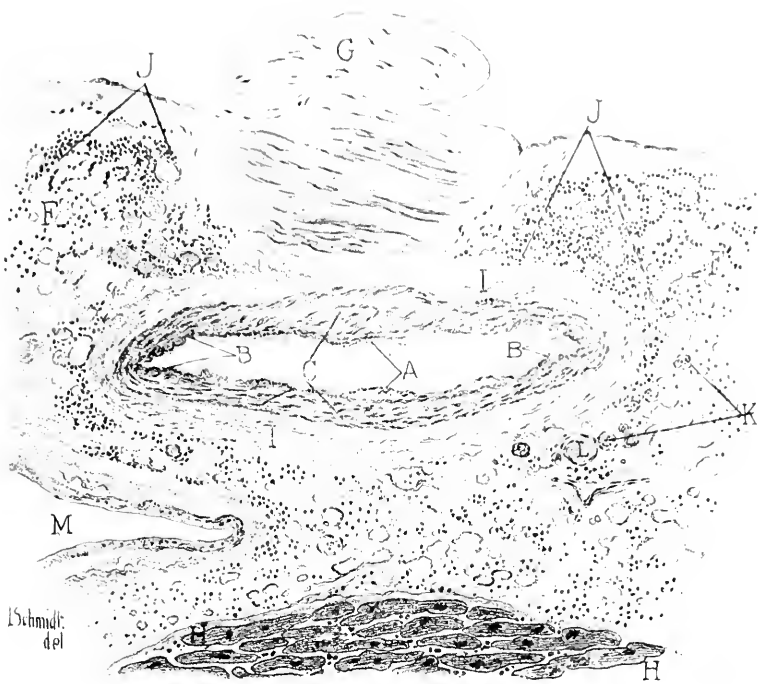

FIG. 1.

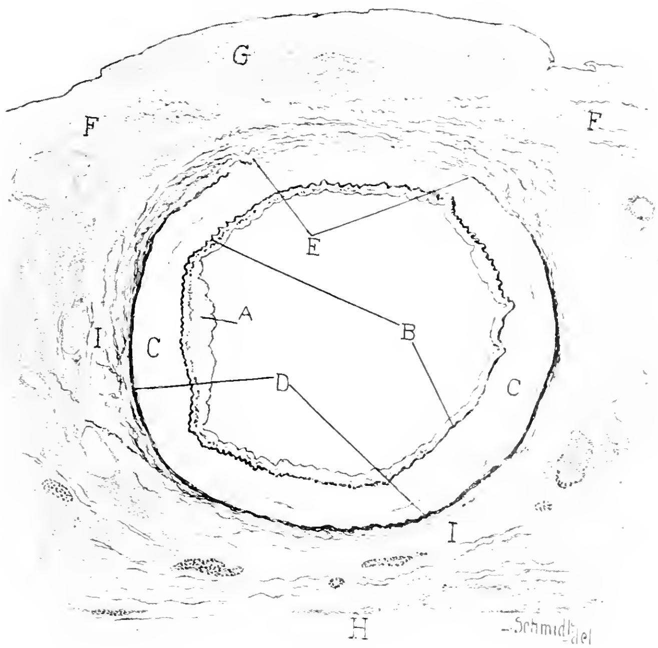

FIG. 2.

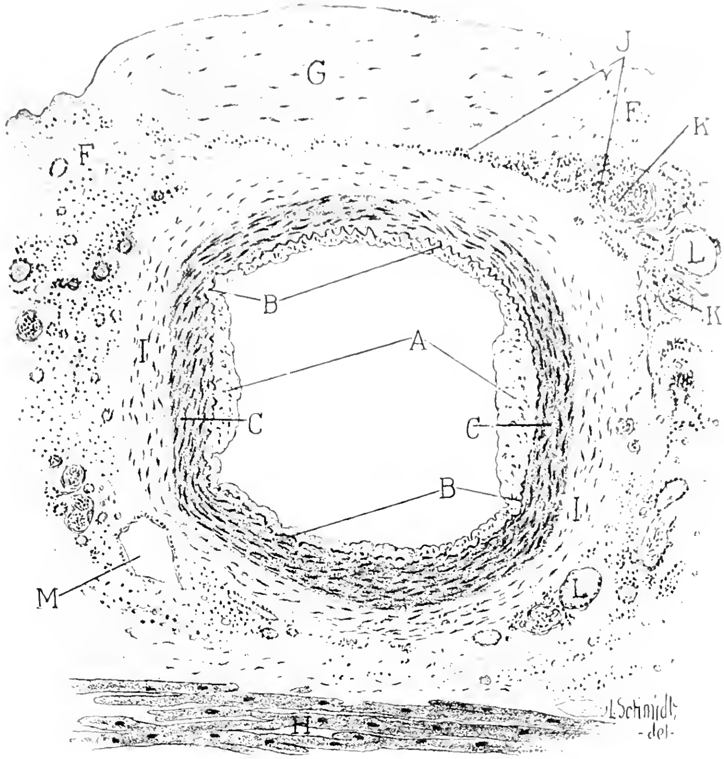
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FIG. 1.

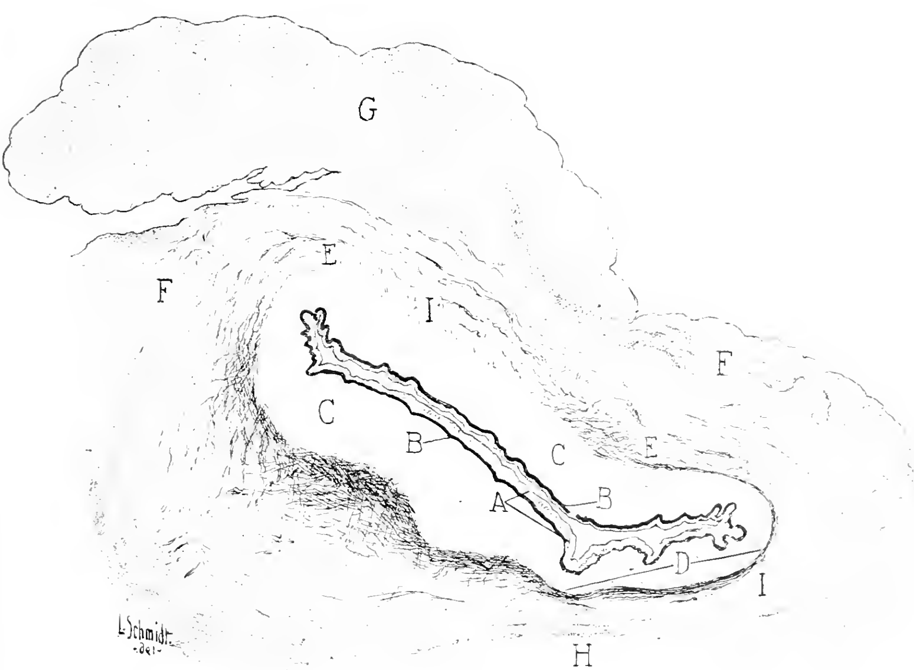
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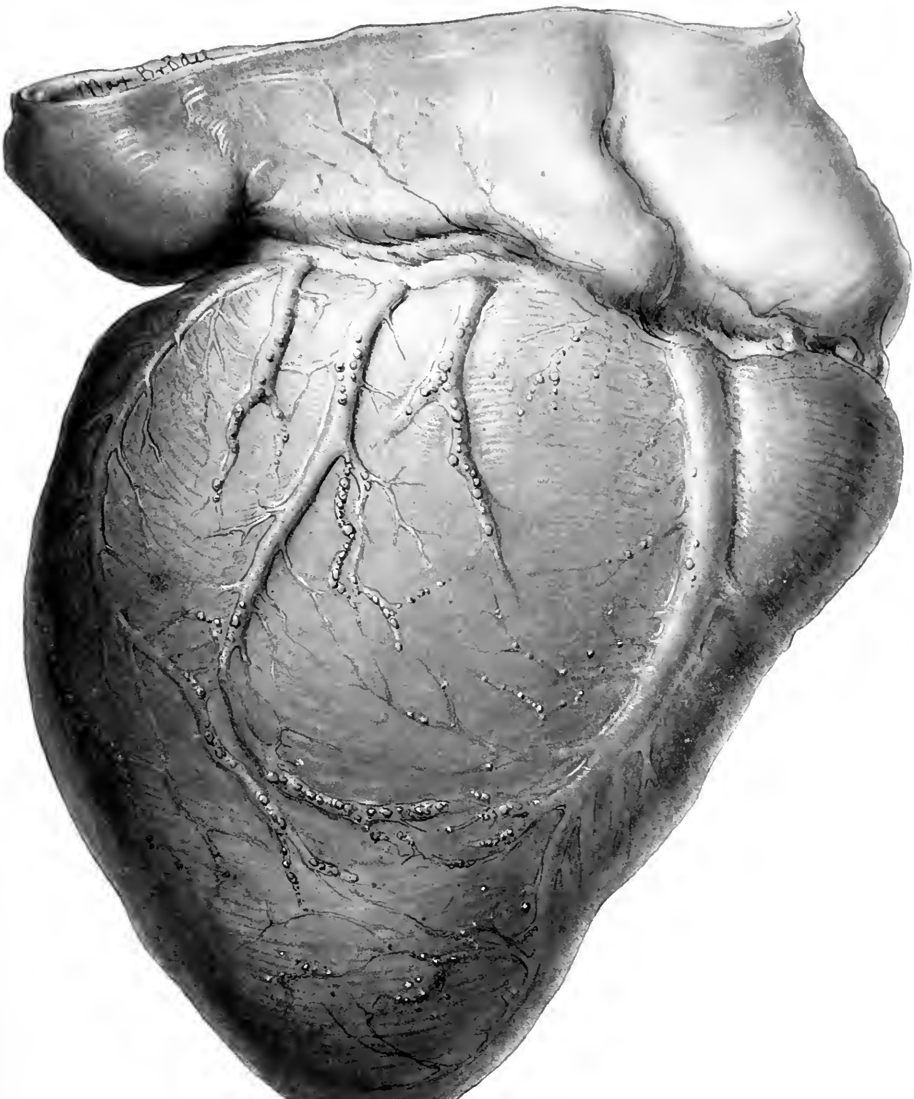
FIG. 2.



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THE ORGANISM IN A CASE OF BLASTOMYCETIC DERMATITIS.*

BY LUDVIG HEKTOEN.

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PLATES XII-XV.

Since the description by Gilchrist,† in 1894, of blastomycetic dermatitis, and the report by Gilchrist and Stokes‡ of the second case, two more instances have been recorded, one by my assistant, H. G. Wells,§ which like the two previous cases resembled lupus vulgaris clinically, and the other and fourth by R. Hessler.|| The latter's case began as a pimple, which gradually enlarged and formed a small abscess, from the contents of which a blastomyces was cultivated. After evacuation the abscess healed, but at the time of the report new foci were making their appearance in the neighborhood of the old scar. The only other of the four instances of blastomycosis of the skin, in which the organism has been isolated in pure culture, is that of Gilchrist and Stokes, who record full observations on the cultural, morphological, and

* Read by title at the meeting of the Association of American Physicians. Washington, D. C., May 1-3, 1899.

† *Johns Hopkins Hospital Reports*, 1896, i, 269.

‡ *Johns Hopkins Hospital Bulletin*, 1896, vii, 129; *Journal of Experimental Medicine*, 1898, iii, 53.

§ *New York Medical Journal*, March 26, 1898.

|| *Indiana Medical Journal*, August, 1898.

pathogenic properties of the Blastomyces dermatitidis, which is the name they gave the fungus in their case.

It is now proposed to describe briefly the results of the histological and biological examination in the case of blastomycetic dermatitis recently under the care of Professors Hyde and Bevan, in the Presbyterian Hospital, of Chicago, so as to add to the number of instances illustrative of the action of pathogenic blastomycetes—microorganisms which of late are attracting considerable attention.

I am under many obligations to Professor Hyde, who saw the case first and referred it to Professor Bevan, for the privilege of making the following abstract of the clinical history:*

R. B., by occupation a day laborer, 56 years of age and married, presented himself for treatment September 20, 1898. He was a native of Holland; at 18 years of age he left for the East Indies, where he entered the Dutch army, serving in the East. He was then taken ill with some form of fever, which resulted in his leaving the East Indies for his native land. He remained in Holland for eight or nine years after, and came to America in 1892.

His present malady began four years ago by the development of a small reddish spot in the right leg, the disorder gradually extending over its anterior part. For this trouble he underwent some operation in the Dearborn Hospital; he was also treated at the Hahnemann Hospital. The result was the production, in the region named, of a thin, broad, cicatriform tissue, insensitive, and in places scaling.

One year ago the left thumb and wrist were found to be involved in some morbid process, which produced by gradual extension from the point of original implication a reddish patch, well defined in outline, with a bluish-red areola and a decidedly verrucous aspect. This patch furnished a moist and sticky exudate. The subjective symptoms were a sense of soreness and burning, at times decided pain and pricking sensations. The pain often radiated up the arm, so that both the elbow and shoulder of the affected side became sensitive. The fingers became partially flexed toward the palm; the little finger was thus permanently flexed.

* The clinical aspects of this case are fully dwelt upon in an article by Hyde, Hektoen and Bevan (A Contribution to the Study of Blastomycetic Dermatitis, *British Journal of Dermatology*, 1899, xi). Here is also a brief report of a new case of blastomycosis of the skin of the leg which I discovered in a museum specimen.

He suffered from headaches, which were frequent and severe; from constipation, and from inappetence. His sleep was poor. His height was 5 feet 9 inches; weight, 98 pounds, a decline from his average weight of 145 pounds. He used tobacco in moderation but no alcoholic stimulants, though he had once been a heavy drinker.

His father died at the age of 92, in good health; his mother at 45, of consumption. There were three brothers living, one dead from consumption; three sisters were dead of consumption, at 21, 16, and 48 years of age, respectively. He had had six children; four were living and in good health, two were dead, one at 39 months, the other at 2½ years, from causes unknown.

When examined by Prof. Hyde the region of involvement of the right leg extended in an irregular oval, its long axis parallel with the axis of the extremity, from the lower margin of the upper third of the anterior surface of the limb quite to the lower border of the lower third, its lateral wings barely perceptible when the posterior surface of the leg was viewed from behind. The encroachment upon the calf was about equal on the two sides, the outer wing slightly more elevated than its fellow. This entire surface was covered with a thin, papery substitute for the normal epidermis, which furnished a slight scale.

The dorsal aspect of the proximal phalanx of the right thumb and of the wrist, with a portion of the posterior face of the lower third of the forearm, were involved in a single connected patch of morbid tissue, dull-reddish in hue, sharply defined in outline and having a peculiar bluish-red border extending about 4 mm. away from the edge of the affected area. The body of the patch was made up of an elevated mass of infiltration, with a verruciform development of fine, pin-head-sized papillæ projecting externally and moistened by a glutinous secretion. Individual elements of this warty growth were readily distinguished by the eye. The palm was completely spared. There was entire absence of other lesions, such as pustules, vesicles, crusts, or the scar-like tissue exhibited on the leg. No other signs of disease were discovered.

For the purpose of further examination and study of the lesion presented by the patient, he was placed in the Presbyterian Hospital, under the charge of Professor Bevan who, on September 27, 1898, excised a small piece of the upper margin. Frozen sections, stained with hæmatoxylin and eosin, showed extensive epithelial proliferations and cell infiltrations in the corium; in the new-formed epithelial masses were numerous miliary abscesses, in one of which I found two round bodies, composed of an outer bluish membrane, an inner colorless and hyaline layer,

and faintly stained granular contents with a few blue bodies like cocci. A diagnosis of blastomycetic dermatitis was accordingly made.

Soon afterward Professor Bevan placed the patient on iodide of potassium, with the result that material diminution in the size of the patch occurred, and such marked amelioration of all the symptoms that the question of operative interference was for the time set aside. The patient had been taking the drug named before he first came under Professor Hyde's observation, but probably not in such doses as those administered at the hospital.

Microscopical Examination.—Portions of the bit of tissue excised September 27 were fixed in absolute alcohol. On October 6 Professor Bevan, at my request, excised a long, narrow strip from the centre of the lesion on the back of the hand, the clinical appearance of which now indicated rapid healing, especially at the margins. Pieces from this strip were fixed in absolute alcohol, sublimate and Zenker's fluid. Paraffin sections were stained with hæmatoxylin and eosin, Gram's method preceded by carmine, and alkaline methylene-blue, with or without counterstain with eosin.

The histology of the skin lesion may be summarized as follows: It can be said at the outset that the histological appearances in this case are identical with those described by Gilchrist and by Wells. In fact their descriptions would answer very well for this case also.

The surface of the skin is covered by a horny layer of varying thickness, together with debris, polymorphonuclear leucocytes, blood discs and bacteria, especially bacilli that take Gram's stain. In places small abscesses have broken through the horny layer, which lies directly on the granular or prickle layer. The striking feature in the sections is the great hyperplasia of the epithelium, very similar to rapid carcinomatous proliferation, and in the form of variously shaped and branching downgrowths of from 3 to 5 mm. in extent, which start from the interpapillary processes principally and cause great distortion of the papillary layer. In the larger columns of epithelial cells the central parts may show degeneration as well as distinct hornification. Isolated epithelial whorls, more or less completely hornified, also occur. The epithelial proliferations are generally separated from the fibrous tissue of the corium by a quite distinct layer of cylindrical epithelial cells, next to which occur typical prickle cells. Occasionally karyokinetic figures occur in the epithelial cells.

Scattered throughout the entire epithelial portion are polymorphonuclear leucocytes, which occur between as well as within the cells. Nu-

merous focal collections of leucocytes, which when larger form miliary abscesses, are found in all parts of the thickened epithelium, in the deep prolongations, near the surface as well as directly underneath it, and at times they have broken through externally. The contents of these characteristic abscesses consist of leucocytes, with more or less nuclear fragmentation, desquamated epithelial cells, detritus of horny material, red blood corpuscles, and the organism peculiar to the process; in some are also multinuclear giant cells of the tuberculous type. The epithelial cells around the abscesses seem to play an entirely passive role and to be flattened out by the pressure of the abscess contents.

The connective tissue between the epithelial downgrowths presents typical acute and subacute inflammatory changes; the vessels are congested, leucocytes are seen in the act of migration, there are hæmorrhagic extravasations as well as œdematous districts. The cell infiltration is marked in many places, but there are but very few miliary abscesses and no organisms have been found in the corium; a few giant cells are present; endothelioid cells, plasma cells, and polymorphonuclear leucocytes make up the principal part of the infiltration; the mast cells are rather numerous. In places young connective tissue has formed. Occasional typical hyaline bodies, mostly as mulberry-shaped masses, are present. In one instance a large cell with a nucleus like that of plasma cells was filled with small hyaline spheres.

As to the appendages of the skin present it would seem that they play but a passive role; the coils of the sweat glands are crowded apart by the inflammatory infiltration, and the hair follicles are the seat of a marked hyperkeratosis.

The organisms in this case do not seem to be present in the tissues as numerously as in the cases previously reported. This impression is gained after carefully looking over a large number of sections from various parts of the area involved. All the mature organisms seen occur in the miliary abscesses in the epithelial proliferations (Figs. 1 and 2, Plate XII), most often singly, sometimes in groups of from two to four; they are invariably situated outside of the cells; not even the giant cells in this case contain any parasites. The parasites, when not budding, are round or oval, and about 10 to 12 mikrons in diameter; they are surrounded by a homogeneous capsule, from which the finely granular protoplasm is separated by a clear zone of varying width; in some the protoplasm contains a vacuole of varying size, which in a

few instances occupies the larger part of the cell, crowding the granules closely together inside the clear zone, which becomes indistinct; in a few organisms the outer capsule contains oblong thickenings. Budding bodies in varying stages are present; the granular protoplasm pushes the capsule and clear zone before it, forming an oval bud which grows larger and eventually separates from the mother organism. Methylene blue gives the best stain of the parasite, the capsule assuming a deep blue color, the protoplasm a lighter blue. There are no red granules in the parasites when stained in this way, as described by Gilchrist.

*Cultures.**—Smears of soft material from the bit of skin first excised contained a few bacilli and a very small number of round bodies similar to those found in the sections. Three glycerine-agar and three blood-serum tubes were inoculated. The resulting colonies consisted of a bacillus, mixed with a few large round bodies; attempts at isolation of the latter by means of the plate method failed. Two attempts at cultivating yeasts from the hand yielded pure growths of a pathogenic bacillus which belongs in the pseudo-diphtheria group. Numerous cultures were also made from the strip of skin removed October 6; small pieces of tissue were rubbed over the surface of potato, glycerine-agar and glucose-agar; inoculations were also made in Pasteur's fluid. After 24 hours there was a heavy growth upon all the solid media, consisting of a comparatively few large clear bodies and bacilli. Isolated by the plate method the bacilli proved to be pseudo-diphtheria bacilli already found, and an unidentified bacillus; the plates also showed some colonies which consisted of round bodies of variable size; in the subcultures these bodies were so closely and constantly associated with small coccus-like organisms that for a time grave doubts were felt as to the purity of the growth. In subcultures on glycerine- and glucose-agar there were noticed after several days colonies with two distinct shades of color, namely a creamy yellow and an almost pure white. Now, from the yellow part the smears showed only small, round or oval, often apparently budding and deeply stained organisms, often not larger than large cocci, while the white colonies consisted of much larger, mostly quite clear round bodies, varying in diameter from 7 to 10 mikrons.

Inoculations of the smaller organism in the yellow colony on fluid media were followed in two or three days by the growth of a fine film on

* I am indebted to Mr. H. E. Davies for the successful isolation of the blastomyces in pure culture.

the surface, which consisted of small bodies, while the slight deposit at the bottom contained mostly larger forms. Inoculations on bouillon with the larger form gave the same results. Cultures of the smaller organisms on glucose- and glycerine-agar at room temperature developed both forms, smears showing long chains of more or less deeply stained small bodies which were closely connected, and at one end of the chain there would often be one or more of the large clear organisms. It was also soon noticed that when a culture of the larger form became dry the organism seemed gradually to become smaller. It was therefore concluded that it concerned one and the same organism, which in the course of artificial growth appeared in various sizes.

This organism grows readily on all of the following media, the extent and rapidity of growth corresponding roughly to the order in which they are named, beginning with the most favorable: beerwort-agar, glucose- and glycerine-agar, Löffler's blood-serum, potato (alkaline), glucose- and glycerine-broth, lactose-, dextrose-, and saccharose-broth, agar-agar, gelatine, milk, and Pasteur's fluid.

Agar and Gelatin Plates.—On glycerine-agar plates pin-point-sized and even larger colonies develop in 24 hours at 35° C.; they are roundish and oval, rather coarsely but evenly granular and very light grayish in color. With a moderate magnification the individual yeast bodies are readily distinguishable, especially at the margins, where budding forms often occur (Fig. 3, Plate XIII). As the colonies become larger the central part turns more opaque; the surface colonies are larger than the deep.

The deep colonies on gelatin plates show the individual, clear, circular bodies very distinctly, while the surface growths are smaller, irregular in outline, more opaque, and composed of much smaller organisms. In the hanging drop colonies of gelatin small, opaque buds are seen between the large, clear organisms. Generally the process of budding results in the growth of small round colonies, but sometimes peculiar, long outgrowths of two or more quite parallel rows of bodies develop, so that the colony becomes more or less club-shaped.

Hanging-drop cultures of bouillon show the multiplication by budding very well, and also the structure of the organism (Fig. 4, Plate XIII).

Agar.—The organism grows most luxuriantly upon 5 per cent beer-wort-agar. On slanting wort- and glycerine-agar there develops in 24 hours an extensive, grayish or yellowish-white growth, without any special characteristics and which spreads itself over the surface and, especially in the case of the beer-wort-agar, gradually creeps in between the

medium and the glass. With time the growth becomes perhaps more purely white; it remains quite soft and smooth on the surface. In from 8 to 14 days quite abundant, fluffy and feathery masses have grown down into the agar from the under surface of the superficial growth; this is more marked on the beer-wort-agar. These peculiar feathery or downy outgrowths are roundish or cone-shaped, the large ones being as much as 3 mm. in diameter; they are very much like the downgrowths characteristic of many varieties of ray fungi.

In stab cultures the surface is soon covered by a spreading, slightly raised smooth layer. The growth along the needle track becomes quite thick and presents irregular, nodular, sometimes almost polypoid protuberances, which give it a peculiar twisted appearance. In a few days this knobby trunk presents delicate lateral branches springing from various parts of the circumference and passing out into the medium at right angles to the stem, after the fashion of the branches of certain trees. These feathery, often extremely fine, lateral outgrowths resemble very much the tree-like branchings of stab cultures of many forms of actinomyces.

On agar-agar the organism produces a rather light yellowish-brown pigment, which smears show to be in the form of small granules that occur about and upon some of the larger clear bodies. This property of pigment production is not observed upon any other media and develops, to a moderate degree, on transplantation upon plain agar from whatsoever other medium it may be, but it is not absolutely constant.

Gelatin.—The growth on gelatin is rather scanty. There develop a flat, thin, pearly surface layer and an irregularly granular tapering growth along the needle track, with peculiar lateral outgrowths consisting of delicate tufts, straight or branching threads of varying length and thickness; some of the threads break up into an extremely fine terminal network at some distance from the main stem. Gelatin is not liquefied.

Serum.—On Löffler's serum the growth is quite extensive.

Potato.—On alkaline potato there forms in 24 hours a slight yellowish-white, raised growth of considerable extent; with time this increases materially, the margins being rounded and irregularly wavy. On acid potato the rate of growth is much slower.

Bouillon.—In bouillon there forms a granular, rather coarse, sometimes a little stringy sediment and gradually small granular masses appear scattered throughout the fluid, which at first is quite clear. Sometimes a clear film develops on the surface.

Milk.—Moderate growth occurs in milk, without any change in reaction and without caseation.

Fermentation Tests.—The organism grows fairly well in the aerobic bulb of fermentation-tubes containing lactose-, dextrose-, and saccharose-bouillon, made from sugar-free bouillon, prepared according to Theobald Smith's formula,* but without fermentation. It also grows to a limited extent in Pasteur's fluid. It does not produce indol.

Reaction to Oxygen.—The organism does not grow in Buchner's jars.

Temperature.—The thermal death-point is 54° C., an exposure to this temperature for two minutes killing the organism. It is not killed by freezing; cultures exposed to a temperature just above zero for two weeks and then frozen solid for two weeks, on inoculation gave rise to typical new colonies.

Relation to Potassium Iodide and the Pseudo-diphtheria Bacillus.—The rapid improvement of the local lesion under the use of iodide of potassium suggested the advisability of determining the effect if any of the presence of this drug in the media inoculated. It was found that a 1 per cent iodide of potassium bouillon furnishes just as favorable a medium for the growth of the organism as ordinary broth.

It also occurred to us that possibly the mixed infection with the pseudo-diphtheria bacillus referred to might play some role in the rapid healing of the lesions. The blastomyces was found to grow unhindered in the presence of the bacillus in the same media; it develops just as rapidly in filtered two weeks' old bouillon cultures of the pseudo-diphtheria bacillus as in the ordinary broths.

Morphology.—The size, shape, and structure of the organism as it develops in artificial culture vary somewhat in the different media and with age. Some reference to this variability has already been made in connection with the account of the early efforts to obtain pure cultures. Fresh specimens mounted in salt solution, from the culture of glycerine-agar, show a highly refractive organism with a doubly contoured membrane.

The organism is not destroyed by caustic potash. It stains readily with the common aniline solutions, but the stain is rather deep and in many cases too diffuse and the clearest pictures are obtained by a rather prolonged staining—15 to 30 min.—of carefully made films in a .5 per cent solution of methylene-blue and then washing well with water. The films are made by suspending a small quantity of the culture in a drop of physiological salt solution or of distilled water and drying. This stain brings out the different parts of the cells whereas other methods stain diffusely.

* *Journal of Experimental Medicine*, 1897, ii, 546.

Large bodies, such as seen in the tissues of the hand as well as in those of infected animals, are not constant in the cultures. However, as the organism begins to grow on most of the common solid media and under favorable circumstances, it generally presents an outer well-defined but thin membrane, which is separated from the protoplasm proper by a clear and transparent zone. The size varies between 7 and 12 μ. The form is round or oval, sometimes polygonal on account of mutual pressure. Budding forms are very frequent and occur in all stages. The process of budding seems to begin with the appearance of a small projection of the endosporium, which pushes the transparent zone and outer membrane in front of it; very soon these layers enclose the new bud fully and the point of attachment to the mother cell may be either flattened or, later, drawn out into a slender pedicle. When the buds are small it is not always possible to recognize the various parts mentioned. In the fairly well developed bodies the endosporium is vacuolated and, generally, finely granular; not rarely some of the granules may be coarse, of varying size, and deeply stained, sometimes producing appearances that resemble the presence of a nucleus, but this is not constant. The vacuole, which may be large or small, is as a rule centrally located. Sometimes there is no vacuole; this is generally the case in young, small buds and newly separated bodies. As the culture becomes older the number of really large bodies with large vacuoles seems gradually to increase; the protoplasmic granules, which also become larger, are then either crowded to one side or arranged as a rim around the vacuole; in many of these bodies the transparent zone is no longer distinctly recognizable. Such large bodies are surrounded by numerous much smaller ones, sometimes vacuolated, sometimes not. In some preparations these bodies seem to lie in a gelatinous material. In a glycerine-agar culture five weeks old or more large bodies are usually quite numerous. In some cultures the size of the cells, the extent of vacuolation, etc., are quite uniform, in others rather variable. The uniform size, regular and characteristic form and large vacuoles of the cells in cultures obtained from an extensive and richly cellular exudate produced by inserting a quantity of yeast-cells into the anterior chamber of a rabbit's eye have persisted unchanged through many generations grown on glycerine-agar.

Large bodies with huge vacuoles are also prone to form in cultures on Löffler's blood serum (Fig. 5, Plate XIII, and Figs. 6 and 7, Plate XIV).

With Gram's stain some organisms remain deeply and diffusely stained while others are partly decolorized, frequently showing, however, three or more deeply colored granules at or upon the margins of the bodies.

By means of stained microscopic sections of the stab cultures in glucose-agar and gelatine it is readily determined that the peculiar lateral outgrowths and branchings observed in these cultures do not depend upon the formation of mycelium but are composed of budding round forms only. This peculiar way of growing is therefore identical with the development of the curious club-shaped colonies in the gelatine hanging drop.

In liquid media, especially bouillon and Pasteur's fluid, as well as, but to a much less extent, on potato and other solid substance, the organism may be comparatively small. It appears that the buds become separated while still minute. Not rarely a parent cell is found surrounded by a number of minute free as well as attached buds, many of which are so small as to resemble cocci. Now, these small bodies, which, as a rule, stain diffusely and homogeneously, may give rise to succeeding generations of small organisms, varying in size from 1 to 5 μ. Repeated budding without complete separation may give rise to chains and groups of various lengths and sizes. Certain parts of smear preparations from cultures in Pasteur's fluid and bouillon may resemble very much those of a medium-sized micrococcus, except that the yeast fungus may present rather pronounced variations in size (Fig. 8, Plate XIV). When the minute forms cluster closely around large, vacuolated bodies the first impression that it concerns a mixed culture is certainly not surprising.

In some cultures a distinct mycelium develops. This is most noticeable in Pasteur's fluid and bouillon, but mycelioid growth-forms may occur to a very slight degree in all media, *e. g.*, plain agar. The formation of mycelium is due apparently to a gradual elongation of individual organisms of the smaller type, resulting in the early stages in irregularly cylindrical-shaped bodies which later grow out into either curved or fairly straight, rather thick rods of varying lengths or, more rarely, form long coiled threads. Buds may arise from any part of the mycelium and they may be sessile or pedunculated. When buds separate from the ends of a rod and grow in length or when all the members in a chain of organisms begin to elongate, a segmented mycelium is formed: the appearance between the ends of two segments of new buds which also grow out long, or the dislocation of a segment from its position in the mycelium, may give rise to quite typical false ramifications. The cylindrical masses, the rods and threads vary in thickness, the average being about 5 mikrons. For the most part the substance of the mycelium is solid and dense, staining diffusely, but parts occur in which there is a membrane enclosing an empty space containing in places finer or coarser granules (Fig. 9, Plate XV).

Cultures on agar-agar are frequently characterized by the production of a granular, yellowish-brown, at times also reddish, pigment. Here are found medium-sized typical bodies, quite a few oblong and elongated narrow, diffusely stained forms but no typical mycelium, and also a considerable number of round bodies covered and surrounded by yellow or yellowish-brown pigment granules which are quite uniform in size (Fig. 10, Plate XV). There is no pigment about the long forms. In the early stages of pigment formation the granules appear in the immediate vicinity of the outer capsule, both within as well as outside it. As the amount of pigment about an organism increases, the endosporium disappears—a fact pointing to a pigmentary degeneration of these bodies. The rather limited growth on plain agar has already been mentioned.

Animal Experiments.—1. A minute fragment of the first bit of skin was inserted into the peritoneal cavity of a guinea-pig, which died a month later, greatly emaciated. There were no changes at the point of inoculation or in the peritoneal cavity, cultures from the small amount of clear fluid in which remained sterile. The organs contained in pure form the pseudo-diphtheria bacillus, previously isolated from the hand. The liver was extensively necrotic and cirrhotic, but these changes are attributable to the bacillus solely.

2. A white rat was inoculated subcutaneously with 1.5 cc. of a bouillon culture. A small local abscess formed. The animal died in ten days. The abscess cavity contained a few cc. of a whitish-yellow viscid pus, from which the blastomyces grew in pure culture. The internal organs were sterile.

Microscopic examination of the abscess wall shows a thin capsule of young fibrous tissue covered by a quite thick layer of pus cells and nuclear detritus, among which rather small, doubly contoured, circular bodies resembling blastomycetes are seen. The liver contains a few minute foci of typical granulation tissue composed of plasma cells, endothelioid cells, small giant cells and polymorphonuclear leucocytes, but it is not possible to find any typical organisms in these areas. There are quite a number of small, similar foci in the lungs, especially around the bronchi, and in the giant cells of these areas occasional rather typical, round bodies are observed. The other organs are normal.

3. A gray mouse died five days after receiving 1 cc. of a bouillon culture subcutaneously. The abscess which had formed contained the organism in pure culture, while the internal organs were normal.

4. A medium-sized rabbit died 48 hours after subcutaneous inoculation of 2.5 cc. of a bouillon culture. Cultures from the internal organs

remained sterile. There was an extensive coccidiosis of the liver. There were numerous minute foci in the lungs composed of epithelioid and giant cells, as well as leucocytes with considerable nuclear degeneration. In some of the giant cells were circular bodies resembling the organism injected, as well as small, round bodies that stained rather diffusely with methylene-blue, presenting a faint peripheral transparent zone. (There is no record of the condition at the site of the inoculation.)

5. A white rat received 1.5 cc. of a bouillon culture. Viscid, yellow pus formed about the point of inoculation and the organism was reclaimed in pure culture. The animal died five days after the injection. The internal organs contained the Staphylococcus albus.

6. A gray mouse was inoculated subcutaneously with 1 cc. of a bouillon culture. It died in five days. The small abscess that had formed contained the organisms, as well as Staphylococcus albus: the latter organism was present in all the internal organs.

7. A small part of a colony on agar was inserted into the anterior chamber of the left eye of a large male rabbit. There gradually developed a hypopyon, with softening and threatened destruction of the cornea. The animal was killed after two weeks. The internal organs, microscopically healthy. The anterior chamber filled to distention with a semisolid, yellowish-gray material, which when spread out on cover slips and treated with hydrate of potassium contained numerous spherical bodies. Inoculations with this material on glycerine-agar gives a rich and pure growth of the blastomyces. Cultures from other organs sterile. Sections of the eye show the anterior chamber to be filled with cellular detritus, among which are a number of round, deeply stained bodies surrounded by a rather faint halo, but no distinct outer membrane. The lens is largely softened and the iris is the seat of a diffuse cellular infiltration. The iris also contains rather small, deeply stained, round bodies which at times are surrounded by minute, hyaline-like formations that take the methylene-blue stain very intensely.

8. A small bit of a yeast culture on glycerine-agar was inserted into the anterior chamber of the eye of a large white rabbit. The anterior chamber rapidly filled with a white exudate, the cornea became opaque, and a mucopurulent conjunctivitis developed which soon ceased. Three weeks after the inoculation the cornea had become quite opaque and vascularized, and the anterior chamber contained a large, roundish, yellow mass which has since slowly enlarged. At present, February 27, 1899, the eye is of about normal size, apparently blind, and the yellow growth does not show any signs of diminution. The rabbit seems to be in good general health.

9. A guinea-pig received 1 cc. of a bouillon culture in the abdomen. It remained well, and was killed five weeks later. There were numerous small, grayish-yellow, translucent areas in the lungs; the other organs were healthy. Cultures from all the internal organs remained sterile. Microscopically the areas in the lungs consisted of foci of granulation tissue without any giant cells, but with extensive nuclear fragmentation. Only a few round bodies were to be found, after prolonged search in numerous sections, stained with methylene-blue, as well as by other means.

10. A white rat received 1 cc. of a bouillon culture in the abdomen. It died thirty-four days later. The lungs contained pin-head sized and larger areas of a grayish, rather soft appearance. Smears from these, treated with caustic potash, showed numerous large, round, doubly contoured bodies. The microscopic sections showed extensive bronchopneumonic areas of an inflammatory tissue with marked nuclear fragmentation and quite a number of large round bodies, which stained diffusely. (Unfortunately no cultures were made in this case.)

11. A medium-sized rabbit was injected with 0.5 cc. of a bouillon suspension through the ear vein. Considerable swelling resulted, which healed after three weeks with marked deformity. The animal, which seemed well, was killed 37 days later. The lungs contained a few small nodules; the liver was the seat of a marked coccidiosis. Cultures from all the organs remained sterile. Sections from the lungs show miliary foci of young granulation tissue, but typical blastomycetes are not to be found.

12. A medium-sized dog was injected with 2 cc. of a bouillon suspension into the peritoneal cavity. The animal remained well and was killed 40 days later. All the organs were normal and sterile.

13. A large black rabbit received into the circulation 4 cc. of a bouillon suspension of a culture on beer-wort-agar. It died during the following night. The lungs were œdematous, the thymus ecchymotic, the liver swollen, soft and mottled; the spleen and the kidneys appeared normal.

Bacteriological Examination.—Smears from the various organs show numerous clear, round bodies not destroyed by KOH; they are most plentiful in the smears from the lungs and kidneys.

Inoculation from the various organs on glycerine-agar give rise to numerous colonies of blastomyces, most numerous in the tubes from the kidneys. The cultures from the liver and spleen also contain the colon bacillus. Only one colony of budding fungi developed in the tube from the heart's blood.

Histological Examination.—The lungs are extremely congested and the capillaries contain many polymorphonuclear leucocytes; in the alveolar walls are numerous small foci of cell accumulation with marked nuclear fragmentation and quite typical blastomycetes are found in the interior of some of these areas of necrosis; the alveoli and bronchi contain a finely granular material in which lie occasional round homogeneous bodies.

The liver and spleen show no special changes.

The kidneys show quite extensive changes in the glomeruli, consisting in a granular disintegration of smaller or larger portions of some of the capillary tufts and of a more or less pronounced general accumulation of granular detritus in the subcapsular space, the cells in the lining of which are generally well preserved. The epithelium of the convoluted tubules is very granular and the lumen is filled with granular material. The vessels are generally congested. Lying in the glomeruli, the intertubular vessels, and in the granular material are a few oval or round bodies about as large as a red blood corpuscle, which stain diffusely with the gentian-violet of Gram's method.

14. February 15, 1899, a guinea-pig, weighing 392 grammes, received in the abdomen 12 cc. of a bouillon culture of the blastomyces heated to 59° C. for 1 hour. It became rapidly emaciated: on February 17 it weighed 340 grammes, on the 20th 285 grammes, on the 22nd 250 grammes: it died on the 22nd. There was marked emaciation but no microscopic lesions in any of the organs or tissues: cultures from the organs and from the heart's blood remained sterile.

The organism above described differs considerably from the Blastomyces dermatitidis of Gilchrist and Stokes, which so far is the only blastomyces of similar origin with which to make comparisons. Hessler's brief description in his preliminary report on the organism isolated by him from a small cutaneous or subcutaneous abscess only contained a few general statements. Our organism grows much more rapidly than the one of Gilchrist and Stokes, the formation of mycelium is not nearly so marked as in their cultures, in which were not seen the peculiar down- and out-growths and lateral branchings nor the pigment formation (on agar-agar) characteristic of the blastomyces now described. Both organisms correspond, however, in their action on gelatin, which is not liquefied, in the non-production of indol, and in the complete absence of fermentation of various sugars. Morpho-

logically they are also quite or nearly alike. Gilchrist and Stokes make no mention of such great variability in the size as observed in our organism.

With regard to the pathogenic action of these two organisms attention may be called to the great similarity, amounting to perfect identity, in the histological changes produced by them in the human skin. The marked epithelial hyperplasia, the diffuse more or less chronic inflammatory processes associated with the formation of giant cells and of the characteristic miliary abscesses in the epithelium and elsewhere in the skin, together with the presence, especially in the abscesses, of the round, double contoured, budding organism, constitute the histological picture of blastomycetic dermatitis as now understood. Gilchrist and Stokes found that the blastomyces isolated by them would produce nodules of a chronic inflammatory nature when inoculated into the dog, horse, sheep, and guinea-pig, whereas white mice and rabbits seemed immune. Our organism may be said to be pathogenic to rabbits, guinea-pigs, white rats, and gray mice. It is probably harmless to the dog, as far as can be judged from our intraperitoneal inoculation of this animal.* Its local action may be characterized as necrotic and leucotactic, associated with or followed by the growth of an inflammatory granulation tissue, corresponding in the latter respect to the majority of the pathogenic yeasts studied by various Italian investigators, by Lydia Rabinowitsch, and others. Its general action may be regarded as slowly toxic, leading after a varying length of time to death from marasmus, and, as experiment No. 14 would seem to show, the dead cultures possess an apparent marantic effect. It corresponds therefore very closely to those varieties of pathogenic blastomycetes which, according to Casagrandi,* pro-

* Since writing the above, the following experiment has been made: On March 22, 8 cc. of a bouillon suspension of blastomycetes were injected into the jugular vein of a small dog, which died greatly emaciated April 17, the autopsy and microscopic examination showing minute foci of granulation tissue throughout the lungs and softened, cellular areas with yellowish contents in the medullary pyramids of the kidneys. The blastomycetes were recovered in pure growth and in large numbers from the lungs and the kidneys. No growth of any kind resulted from the inoculation on glycerine-agar and blood serum of 1 cc. of the heart's blood.

* Ueber die pathogene Wirkung der Blastomyceten, *Centralbl. f. Bakt.*, Abth. I, 1898, xxiv, 754.

duce local necrotic or suppurating foci or permanent nodules and a fatal marasmus. It can probably not be said that the toxic and marantic effects of these forms of blastomycetes have been so pronounced as to have been definitely recognized in the few carefully observed instances of human blastomycetic infection so far recorded, but certainly the marked decline in weight noted by Prof. Hyde in this case is very suggestive of this effect and is therefore in full accord with the experimental observations.

That certain blastomycetes may produce more distinctly suppurative changes in the skin and also elsewhere in man, as well as in animals, has been clearly shown in the now classical case of Busse,* by Hessler's case, and in the experimental study of the question of suppuration from yeasts by Nesczadimenko.† In connection with this, reference may also be made to the instance of refractory subcutaneous abscesses caused by a fungus possibly related to the Sporotricha described by Schenck.‡

To return to the consideration of the blastomycetes studied by Gilchrist and Stokes and by me, it might be regarded as quite clearly demonstrated from some of the foregoing considerations that the clinical and histological pictures of blastomycetic dermatitis, which from these aspects would appear as a distinct entity, may be produced by organisms which differ so much in certain cultural and pathogenic characteristics that they must be regarded as separate, though closely related, varieties. In view of the present unsatisfactory status of classification of the blastomycetes it is desirable to refrain from becoming dogmatic. Casagrandi, for instance, found it quite impossible to classify the blastomycetes upon either morphological or biological grounds on account of the pronounced variability of the individual forms.

* The dermatological aspects of Busse's case have been fully considered by Busche in an article entitled "Ueber Hautblastomykose" (*Verhandl. d. VI. Deutschen Dermatolog.-Congresses*). Unfortunately this article came to my notice too late to be considered at this time.

† Zur Pathogenese der Blastomyceten, *Centralbl. f. Bakt.*, Abth. I. 1899, xxv, 55.

‡ *Bulletin of the Johns Hopkins Hospital*, 1898, ix, 286.

DESCRIPTION OF PLATES XII-XV.

(Photographs by Dr. W. H. Knap.)

Plate XII.

Fig. 1. A miliary abscess in the epithelium of the hand, containing in its upper half a group of three organisms. ×220.

Fig. 2. The three organisms in Fig. 1, more highly magnified. ×1500.

Plate XIII.

Fig. 3. A colony of the organisms on glycerine-agar. ×250.

Fig. 4. The organism as seen in a hanging-drop culture of bouillon. ×1000.

Fig. 5. Vacuolated and solid diffusely stained organisms from glycerine-agar culture. ×1000.

Plate XIV.

Fig. 6. Budding organisms. Gentian-violet. ×1000.

Fig. 7. Large vacuolated and small solid bodies from a blood-serum culture 5 weeks old. ×1000.

Fig. 8. Chains of the minute form. ×1000.

Plate XV.

Fig. 9. The development of mycelium with sessile and pediculated buds. ×1000.

Fig. 10. The development of pigment granules around and upon some of the larger cells in cultures on plain agar. Several elongated forms are present. ×1000.

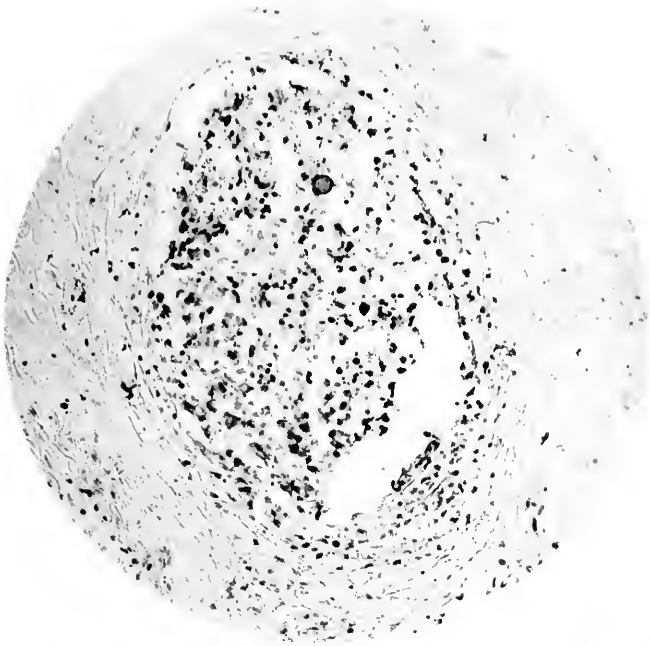

FIG. 1.

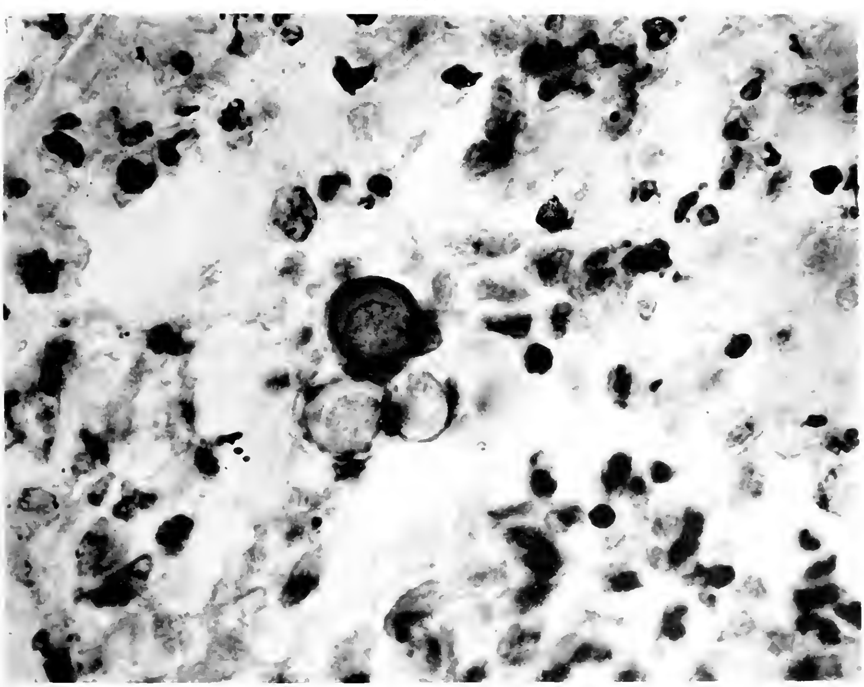

FIG. 2.

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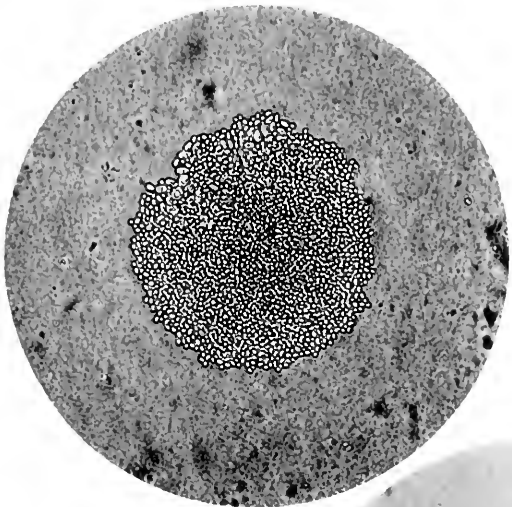

FIG. 3.

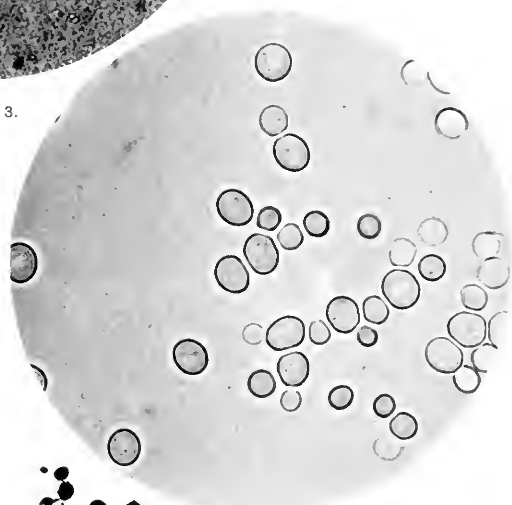

FIG. 4.

FIG. 5.

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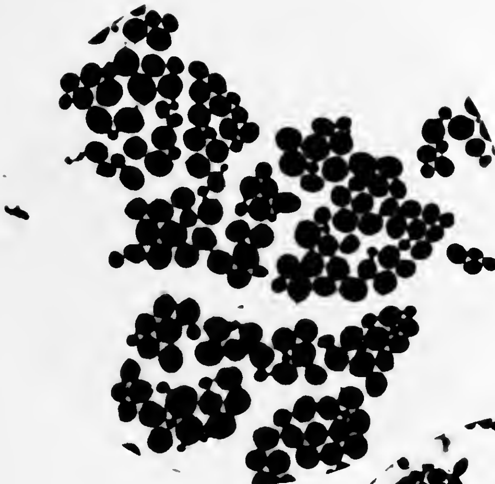

FIG. 6.

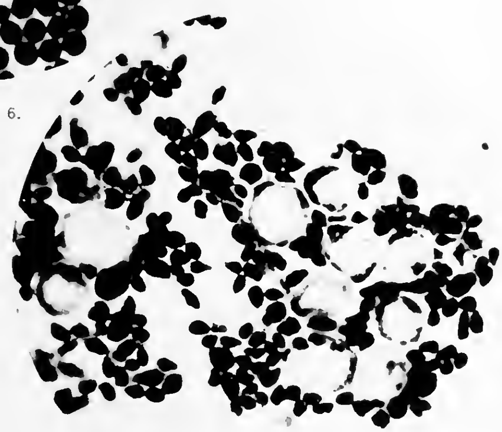

FIG. 7.

FIG. 8.

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FIG. 9.

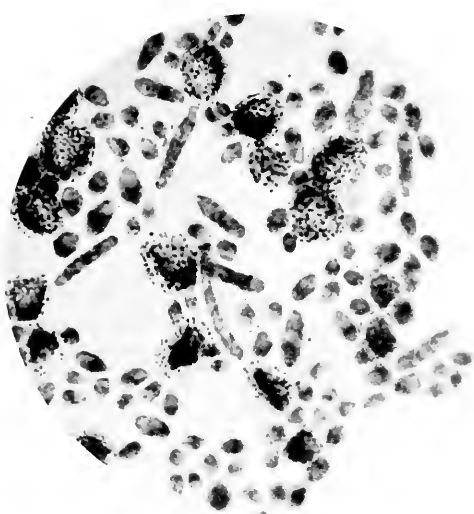

FIG. 10.

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A CASE OF HÆMOCHROMATOSIS.—THE RELATION OF HÆMOCHROMATOSIS TO BRONZED DIABETES.

By EUGENE L. OPIE, M. D.

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Widely disseminated pigmentation of the organs of the body is a conspicuous phenomenon and at autopsy cannot readily be overlooked. The physiology of pigmentation is but little understood. Pathological pigmentation is involved in even greater obscurity. For this reason the following case has been considered worthy of study. It represents, I believe, an intermediate stage between two conditions which, though rare, have elicited much discussion, namely, hæmochromatosis of von Recklinghausen and the bronzed diabetes of Hanot and other French writers. The case to be described occurred in the practice of Dr. Thomas Opie.

Case.—Male; white; aged 55 years. The history which it has been possible to obtain is meagre. The individual, though never very robust, had enjoyed fairly good health. There was no history of alcoholic excess. He was married and had several children. His wife was healthy. For several months he had resided in Puerto Rico and until six or seven weeks before his death was able to continue his work of surveying. The onset of his final illness occurred with symptoms indicative of typhoid fever. When first seen two weeks later, he was evidently very ill. There was elevation of temperature and rose-spots were present upon the abdomen. Deep pigmentation of the skin attracted immediate attention, marked universal bronzing suggesting Addison's disease. Jaundice was not present. The urine at no time during the period of observation contained sugar. The first examination was made four weeks before death. Three days before death the urine was clear, of deep amber color and contained neither sugar nor albumin. The reactions of bile pigments were not obtained. The blood-serum caused the agglutination of the typhoid bacillus. Death occurred with increasing weakness.

Autopsy.—Performed 9 hours after death. The body, 170 cm. in

length, is that of a very thin, sparely built man. The skin over the entire body shows deep pigmentation of a bronzed metallic hue, most marked upon the back of the hands, about the nipples, and upon the penis, where just above the corona the skin has a deep brown color. The conjunctivæ are clear. The abdominal wall contains a small amount of deep yellow fat.

Peritoneal Cavity.—The parietal peritoneum, as well as that of the intestines, shows a varying degree of bluish discoloration. The appendix is fixed behind the cæcum by fibrous adhesions. The enlarged spleen is adherent to the diaphragm.

Thorax.—The lungs are free from adhesions and the pleural and pericardial cavities contain no excess of fluid.

Heart.—Weight 280 grm. The muscle, of a yellowish-brown color, is soft and flabby in texture. The intima of the coronary arteries is roughened by a few yellow, raised patches.

Lungs.—The left lung is everywhere crepitant. Upon the surface of the upper lobe of the right lung are seen several prominent areas, about 2 cm. across, over which the pleura is dulled. The tissue below is firmly consolidated, and on section has a grayish-red, finely granular surface. Several larger areas of similar consolidation are present in the lower lobe. The bronchi are intensely injected.

Liver.—Weight 2270 grm. The surface is of a deep reddish-brown color of peculiar character, resembling that of iron-rust. The surface of the left lobe, more markedly than that of the right, is superficially nodular and puckered, presenting in moderate degree the appearance of a hob-nail liver. On section islands of lighter brown parenchyma, representing one or several lobules, are surrounded by fibrous stroma of a deeper brown color. Sparsely scattered are opaque yellowish-white areas, often 1.5 mm. across. The gall bladder, distended by thin green bile, measures 12 cm. in length.

Spleen.—Weight 700 grm.—The organ is soft and on section the almost diffluent pulp has a deep red color. The Malpighian bodies are prominent.

Stomach.—The mucous membrane shows everywhere a deep greenish-black discoloration, evidently not due to post-mortem change. Along the lesser curvature there are a few areas of relatively normal yellowish-white appearance, but elsewhere the pigmentation is uniform.

Intestine.—The duodenum has a greenish-black discoloration almost equal in intensity to that of the stomach. This pigmentation is not uniform, and upon the areas of lightest color are seen deeply pigmented,

slightly raised nodules about 1 mm. in diameter. The jejunum is less markedly pigmented than the duodenum, while the ileum is of even lighter color, though still preserving a greenish-gray tint. Throughout the lower portion of the ileum, for the most part upon the Peyer's patches, are small round or slightly irregular ulcers with sharp edges and clean base, in which are occasionally seen exposed fibres of the circular muscle coat. Ulceration is most extensive immediately above the ileo-cæcal valve. The colon exhibits moderate greenish-gray discoloration. The distal 1.5 cm. of the appendix is obliterated.

Pancreas.—Weight 170 grm. The organ is of large size, measuring 16 cm. in length, and is very firm in consistence. On section the cut surface has a uniform deep chocolate-brown color. The capsule contains much fat, and extending inward from it are septa of adipose and fibrous tissue.

Adrenals.—Combined weight 10 grm. No abnormality is observable.

Kidneys.—Combined weight 470 grm. The capsule on removal leaves a smooth surface. The cortex of an average thickness of 7 mm. has a red cloudy appearance.

Bladder.—Mucous membrane is normal.

Testicles.—The cut surface has a light brown tint. The prostate and seminal vesicles are normal.

Lymphatic Glands.—In the retroperitoneal tissue near the hepatic vein, behind the stomach, above and below the pancreas, and on either side of the aorta as low down as its bifurcation, are enlarged, moderately firm lymphatic glands. On section they have a uniform, brilliant orange-yellow surface. In the gastro-hepatic omentum are enlarged glands of a similar nature.

Cultures.—From the heart's blood the typhoid bacillus was obtained in pure culture. The same organism was obtained from the liver, gall bladder, and kidney. The Bacillus lactis aërogenes was found in the lung and kidney. The colon bacillus was grown from the pancreas. A culture from the spleen remained sterile.

Anatomical Diagnosis.—Typhoid fever; ulcers in the ileum; acute splenic tumor; cloudy degeneration of the kidneys; broncho-pneumonia. Hæmochromatosis: pigmentation of the liver, pancreas, heart, stomach, intestine, peritoneum, lymphatic glands, skin and testicles. Cirrhosis of the liver. Chronic interstitial pancreatitis.

Histological Examination.—The organs which show the most marked pigmentation are the liver, pancreas, heart and gastro-intestinal canal.

Liver.—Advanced cirrhosis is indicated by the presence of wide bands of fibrous tissue, occupying one-half to one-third of the area of the cut surface and separating the parenchyma into islands which represent one or several liver lobules. These septa, which are densely fibrous and in general poor in cells, surround both the portal spaces and the sublobular veins. There is but little invasion of the periphery of the lobule by fibrous strands, but surrounding the central vein a mass of tissue penetrates the lobule and sends out irregular, radiating projections along the capillaries between the columns of liver cells. The most conspicuous feature of the histological picture is the immense amount of yellowish-brown pigment present, both in the parenchyma and in the interstitial tissue. In the liver cells the pigment is found in varying quantities. When in moderate amount, it is situated in that part of the cell most distant from the capillaries, consequently occupying the central part of of the columns of liver cells. The pigment is deposited in the form of brilliant, brownish-yellow, relatively coarse granules, round or polygonal in outline, and of slightly variable size. The endothelial cells of the capillaries and Küpffer's cells contain granules of similar character. The accumulation of pigment in the newly formed fibrous tissue is even greater than that within the parenchyma. Here it exists in part as fine granules within spindle-shaped cells, of which some are apparently fibrous tissue cells, while others represent the endothelial lining of capillaries or lymphatics. The greater quantity of the pigment is, however, not contained in cells. While this extracellular pigment is of the same yellowish-brown color as that abundant in the cell, the individual particles vary greatly in size, globules being often found whose diameter is two or three times that of a red corpuscle. These pigment particles are as a rule collected into small clumps and between the clumps are at times small round cells.

The hepatic cells which contain a moderate amount of pigment are in general normal in appearance. In specimens stained with methylene-blue the cell protoplasm is found to contain in great abundance granules of varying size and shape taking a deep blue stain. Usually they are angular, but often are rod-shaped and resemble bacilli. A few cells containing fat droplets are occasionally seen at the periphery of the lobule. When, with increased accumulation of pigment, granules occupy all portions of the cell body, basal as well as central, changes in the cell are demonstrable. The nucleus often presents evidence of degeneration. It is smaller, irregular in outline, with less nuclear material present, so that it remains as a pale shrivelled body, visible in some cases only

with careful focusing of the microscope. Other masses of pigment granules maintaining the outline of liver cells contain no nucleus. Representing apparently the final stage of cell disintegration, a mass of pigment granules is found free in the tissue. No longer presenting the outline of a cell, it corresponds in size with the pigment accumulations found in those cells which are most intensely impregnated. Conspicuous in stained sections are groups of pigment masses, which, for the most part maintaining cell outlines, represent often ten or twenty cells and stand out as pigment islands in sharp contrast to the well-stained liver cells adjacent.

In those cells whose protoplasm is almost wholly replaced by pigment much irregularity in the size of the granules is observable. Globules often the size of red corpuscles are found. With further evidence of cell degeneration the size of the globules increases, so that, when final disintegration takes place, there is liberated a mass of granules and globules resembling those found in great abundance in the fibrous tissue. Along the central vein and following the strands of tissue radiating from it are seen masses of pigment whose location indicates that they are transported, presumably along lymphatic spaces, from the interior of the lobule toward the central vein. Abundant accumulation of pigment exists in all parts of the interlobular tissue, both along the course of the sublobular veins and in the tissue about the portal spaces.

The adventitia and media of the hepatic artery and portal vein contain but little of the brownish-yellow pigment. The endothelial cells of the portal vein, however, contain a moderate amount, and within the lumen are seen large flat oval cells containing pigment granules. It seems probable that the majority of these at least are endothelial cells, which after death, possibly in the process of hardening or cutting, have been dislodged from their normal position. The brownish-yellow pigment is not present in the endothelium of the hepatic arteries and in their lumen no pigment-containing cells are seen. Pigment is more abundant in the wall of the sublobular veins. Their endothelial cells contain it in abundance and within their lumen are pigmented cells resembling those found in the portal vein.

Newly formed bile-ducts are numerous in the interstitial tissue. Occasionally in the tissue about the central vein one sees several such structures represented by parallel rows of cubical cells. These ducts contain the brownish-yellow granules in moderate amount. The epithelial cells of the larger ducts contain similar pigment.

The brownish-yellow pigment, whose location has been described, gives

the microchemical reactions characteristic of iron. Treated with potassium ferrocyanide and hydrochloric acid it takes a green color. Aqueous solution of hæmatoxylin gives it a deep black. The reaction with potassium ferrocyanide and hydrochloric acid varies with the time of exposure and with the strength of the reagents. If sections are immersed for an hour to an hour and a half in equal parts of a two per cent solution of potassium ferrocyanide and hydrochloric acid, one part to a hundred parts of water, all the granules within the liver cells assume a green color.

There is present a second less conspicuous variety of pigment differing from that already described both in situation and in morphology. In certain cells in the media and adventitia of both veins and arteries are deposited fine, pale yellow granules of almost uniform size. Some of the cells containing this pigment are elongated and spindle-shaped with rod-like nuclei, while others are large, irregular in shape, and provided with irregular processes. The first are apparently smooth muscle cells, the second connective-tissue cells. The latter are abundant in the walls of the ducts, in the tissue about nerves, in the intervening tissue of the portal space and as a conspicuous layer in the liver capsule a short distance below the serosa. The pigment in these cells does not give the microchemical reactions characteristic of iron, being unchanged by potassium ferrocyanide and hydrochloric acid. In sections stained with methylene-blue these granules take a blue, often bluish-black color, and become very conspicuous. This fact was observed by Buss (8).

Areas of focal necrosis referable to typhoid fever are present. They may represent either a small number of necrotic cells or a considerable portion of a lobule.

Pancreas.—Chronic interstitial inflammation is present. Bands of fibrous tissue separate the lobules which often stand out as isolated islands of parenchyma. Although the most marked increase of fibrous tissue is interlobular, there is here and there an invasion of fibrous strands between the acini. Pigment giving the reactions of iron is deposited in great quantity within the secreting cells and is located, when moderate in amount, in that portion of the cell directed toward the lumen of the acinus—with greater accumulation all parts of the cell body are occupied and, as in the liver cells, globules of large size are then seen. Such a cell may have a palely staining nucleus of irregular outline or a nucleus may not be demonstrable. In the interstitial tissue the pigment is for the most part in groups of granules and large globules, varying much in size. These are apparently masses of pigment set free by the disinte-

gration of cells. About such pigment round cells are often numerous. Pigment is frequently abundant in the cells composing the intertubular cell-groups or islands of Langerhans. These structures are present in moderate number. They have not as a rule their usual position within the lobule, being surrounded by fibrous tissue, which may contain much pigment. At times there is an increase of fibrous tissue along the capillaries penetrating them. Here and there in sections are seen areas which present a peculiar appearance. The cells of one or several lobules or a part of a lobule have lost the alveolar arrangement characteristic of the pancreatic parenchyma. At first sight it may be supposed that this disturbance of the usual structure is artificial in origin, produced possibly in cutting the section, but it has been observed in tissues hardened by a variety of methods and cut after embedding in both paraffin and celloidin. Moreover, the cells of such areas differ from those of the surrounding gland tissue. They are smaller, polygonal in shape. Their nucleus is often smaller and contains more chromatic substance than those adjacent. A conspicuous feature is the abundance of cells containing two nuclei and at times three or even four are seen in the same cell. Mitotic figures have not been observed. The significance of these areas is doubtful; it is possible that they are the seat of cell proliferation and may bear some relation to the increased size of the organ.

Finely granular iron-free pigment, like that found in the walls of the hepatic blood-vessel, is present also within cells of the same character in the walls of the veins and arteries of the pancreas. Pigment of identical characteristics is found in the walls of the larger ducts.

Heart.—Well-marked fragmentation and segmentation of the myocardium is present. Brownish-yellow pigment is deposited in great quantity within the muscle fibres. It has the location of that which is present with brown atrophy of the organ and, when moderate in amount, is accumulated in the neighborhood of the nucleus. All the pigment present does not give the reactions of iron, and in specimens treated with ferrocyanide of potassium or with aqueous hæmotoxylin a considerable amount of unchanged pigment remains. In sections of heart muscle treated with methylene-blue none of the pigment within the muscle cells takes the blue color observed when the iron-free pigment of the walls of the hepatic blood-vessels is so treated. This pigment is apparently that which is found normally in the muscle fibre and is increased in the condition of brown atrophy. The pigment, remaining unchanged after treatment with reagents which demonstrate iron, is greater in amount than that normally present in the muscle fibres. In the

interstitial tissue are seen here and there groups of globules of iron-containing pigment similar to those found free in the tissues elsewhere. The walls of the blood-vessels contain iron-free pigment staining with methylene-blue.

Gastro-Intestinal Tract.—The pigmentation of the stomach and intestine differs from that of the organs previously considered. Here the pale yellow, iron-free pigment predominates and gives microscopic discoloration to the organ. Brownish-yellow, iron-containing pigment is fairly abundant in the epithelial cells of the cardiac glands and is present in very small amount in the cells of the pyloric glands, while an occasional clump of granules is seen free in the tissue. The iron-free granules are in cells which have usually the character of smooth muscle fibres, although a few are apparently connective-tissue corpuscles situated between the muscle fibres or in the submucosa. The distribution of the pigmented cells varies in different parts of the gastro-intestinal tract. Throughout the stomach the muscularis mucosæ is pigmented. In the cardiac portion the circular and longitudinal layers are not so affected, while in the pyloric region there is in the circular muscle layer a conspicuous zone of pigmentation immediately below the submucous tissue. The walls of the arteries and veins of the submucosa contain pigment of the same nature. Throughout the small intestine iron-free granules are found in the cells of the muscularis mucosæ. A small amount of pigment is present in the circular muscle layer, but does not form the zone present in the pyloric portion of the stomach. In all parts of the small intestine pigment is most abundant in the cells of the longitudinal layer. The vessels of the submucosa contain numerous pigmented cells. Less pigment is present in the ileum than in the jejunum, while in the colon it is found in still smaller quantity, though it has the same location as elsewhere. The epithelium of the Brunner's glands of the duodenum are conspicuously impregnated with iron-containing pigment, the protoplasm of the cells being almost completely replaced by pigment granules.

Lymphatic Glands.—Several lymph glands were examined. One of these was situated beside the inferior vena cava where it receives the hepatic vein. Lymphatic tissue is almost wholly replaced by masses of brownish-yellow pigment giving the reactions of iron, so that the organ is practically a mass of pigment surrounded by a thickened capsule and penetrated by thickened fibrous septa. Among the pigment granules are scattered lymphoid cells, small groups remaining here and there. The pigment lying within the meshes of the reticulum is almost wholly

extracellular, being in the form of particles which vary greatly in size and resemble the extracellular pigment in the interstitial tissue of the liver, pancreas and heart. It is apparently carried to these lymphatic glands along lymphatic channels in the same way that coal pigment is carried from the lung to the bronchial glands. An occasional fixed tissue cell containing brownish-yellow granules is observed. In the smooth muscle and connective-tissue cells of the blood-vessels and in connective-tissue corpuscles of the capsule iron-free granules are found.

Spleen.—The great engorgement of the blood-vessels and consequent tumefaction explain the fact that in spite of an abundant pigment deposit there is not the macroscopic pigmentation observed in other organs. Iron-containing granules are present in the branched reticular cells of the pulp and in the endothelial cells of the capillaries. Indeed the capillaries on cross-section often form conspicuous rings of pigment-containing cells. In less quantity round and oval pulp cells contain brownish-yellow granules. In the Malpighian bodies pigment is almost entirely absent, and when found occurs in clumps of irregular extracellular globules, resembling those so abundant in the lymphatic glands. Iron-free pigment is not abundant, but is found occasionally in the elongated connective-tissue cells of the trabeculæ.

Adrenal.—Pigmentation is almost wholly limited to the so-called glomerular zone, which forms a sharply defined corona of brownish-yellow color immediately below the capsule. When moderate in amount, the pigment, which gives the characteristic reactions of iron, is situated in that portion of the cell most distant from the capillaries, but as a rule it is present in such quantity that all portions of the cell body are occupied. Degenerative cell changes similar to those observed in the liver and pancreas are associated with the accumulation of pigment. The capsule in places is very slightly thickened and cellular, and contains globules of pigment, apparently set free by the disintegration of parenchyma cells. In the cell columns there is here and there a deposit of pigment granules.

Kidney.—Pigment is present in very small quantity and is limited to an occasional tubule or glomerulus. Here and there the epithelial cells of a convoluted tubule contain pigment granules in great quantity and differ from those of neighboring tubules in being of smaller size and more cubical. The glomeruli often contain pigment granules located apparently in the epithelial cells and occasionally there is a similar deposit in the loop of Henle. The blood-vessels differ from those of the organs more impregnated with iron in containing almost no iron-free pigment. An occasional cell thus pigmented can be seen, but search is required for its demonstration.

Lung.—Pigment is scant. Occasionally within the alveolar septa are found yellowish-brown granules resembling the iron-containing granules in other situations. At times they completely fill an oval cell with an oval nucleus, while again they are extracellular. In many instances it is evident that these cells or free masses lie within the lumen of capillaries. Pigment-containing cells of a similar nature are seen in the larger vessels and it is not inconceivable that in part at least they are endothelial cells of the liver or other organ, which, injured by pigment accumulation, have been dislodged from their position. Within the lumen of the larger vessels pigment granules are occasionally seen in round mononuclear cells very slightly larger than polymorphonuclear leucocytes. Iron-free pigment is not found in the walls of the blood-vessels.

Skin.—In the lowermost cells of the Malpighian layer there is a well-marked increase of the normal pigmentation. The granules here found do not give the reactions of iron nor do they stain with methylene-blue. In the cells of the sweat glands are occasionally found iron-containing granules in small number and between the tubules are connective-tissue cells containing similar pigment. Iron-free pigment is found in very small amount in the walls of blood-vessels of the subcutaneous tissue. Neither pigment granules nor pigment-containing cells are found within the lumen of the blood-vessels.

Testicle.—The cells of the seminiferous tubules contain no pigment. Along the vessels in the angular interstices between the tubules are conspicuous groups of polygonal and oval cells completely filled with yellow granules, apparently the so-called interstitial cells, which normally contain a small amount of pigment. The endothelial cells of the vessels also contain conspicuous granules. The pigment of the interstitial cells remains unchanged after treatment with potassium ferrocyanide and hydrochloric acid, while that of the adjacent endothelium gives the characteristic reaction of iron.

Under the designation "hæmochromatosis" von Recklinghausen (1) in 1889 described a condition of pigmentation affecting various organs. Brown pigment, which he thinks is derived from the hæmoglobin of the blood, is deposited within certain tissues and gives to them macroscopic pigmentation. Cases of intense widespread pigmentation had previously been described by Quincke (2), Tillmanns (3) and Hindenlang (4). Cases of similar pigmentation, associated

with diabetes and cirrhosis of the liver, had been described by Hanot and other French writers.

The anatomical picture of generalized pigmentation drawn by v. Recklinghausen is very clearly defined. Most of the glands of the body have a deep brown color and within their secreting cells are found reddish-yellow or ochre-colored granules. In the liver this pigment is present in the parenchyma cells and in Küpffer's cells. Microchemical reactions prove that it contains iron. A second kind of pigment, distinguishable from the first by the occurrence in finer granules of pure yellow color, is found in the smooth muscle cells of the stomach and intestines, of the blood- and lymph-vessels, rarely in those of the urinary bladder, ureters, and vas deferens. It also exists in the connective-tissue cells of certain localities, for example, Glisson's capsule, the splenic trabeculæ, and the sheaths of blood-vessels. This pigment does not give the reactions characteristic of iron. v. Recklinghausen calls the iron-containing pigment "hæmosiderin," the iron-free pigment "hæmofuscin." The use of these names does not necessarily imply that we have any means of identifying these substances as definite chemical compounds or that we can recognize them when they occur in other situations.

Von Recklinghausen thinks that the hæmofuscin as well as the iron-containing hæmosiderin is derived from the hæmoglobin. In his cases of generalized pigmentation there was an associated cirrhosis of the liver.

Von Recklinghausen studied twelve cases which he regards as examples of local and general hæmochromatosis. Hæmochromatosis he apparently defines as a condition of pathological pigmentation due to the deposition of pigment derived from the blood. Such a definition includes a variety of dissimilar forms of pigment deposit; brown atrophy of the heart, for example, fulfills its conditions, since, as is believed, the ultimate origin of the pigment there found is the hæmoglobin of the blood. Nevertheless it evidently has but little in common with the general hæmochromatoses already described. There is however, a local condition which has been associated with hæmochromatosis by several observers. I refer to pigmentation of the intestine

caused by deposition of fine yellow granules in the smooth muscle cells. Goebel (5) has made a careful study of this form of pigmentation. In adults a moderate degree is almost constant. The amount of pigment present bears a relationship to the age of the individual, so that with increasing age there is an increased pigment deposition. Other influences, however, may play an important part in producing the condition. With wasting diseases, for example tuberculosis and carcinomatosis, there may be an accumulation equal to that present in advanced age. In sixteen of one hundred bodies studied by Goebel the pigment deposit was of sufficient magnitude to cause macroscopic discoloration, characterized by him as rust brown. Hintze (6) has described two cases of similar intestinal pigmentation.

The pigment present in these cases does not give the reactions of iron and agrees in morphology and location with the hæmofuscin of v. Recklinghausen. Hence both Goebel and Hintze conclude that this intense intestinal pigmentation represents the first stage of hæmochromatosis. Hæmosiderin is, however, not present and it is the hæmosiderin, whose wide distribution forms the most characteristic feature of general hæmochromatosis. Indeed, the iron-free pigment has apparently been overlooked in a number of cases of generalized pigmentation, whose description otherwise agrees with the picture drawn by v. Recklinghausen. It is, moreover, as will be shown, with the presence of this iron-containing pigment that are found marked pathological changes in the containing cells. The accumulation of pigment within the smooth muscle cells of the intestine is apparently an accentuation of a physiological process, much more closely related to the brown atrophy of the heart than to general hæmochromatosis. v. Recklinghausen's description represents a condition, presenting no close similarity to any form of local pigmentation with which we are familiar.

The important features of this description are: (1) The presence in the epithelial cells of various glands, notably the liver and pancreas, of an iron-containing pigment. (2) The presence of an iron-free pigment in smooth muscle cells of the gastro-intestinal tract and of the blood- and lymph-vessels and in certain connective-tissue cells. (3)

The association of cirrhosis of the liver with the pigmentation. To this condition, apparently a distinct pathological entity, the term hæmochromatosis should be limited, even though v. Recklinghausen, its originator, applied it to conditions of local pigmentation as well.

Prior to v. Recklinghausen's publication several observers had studied cases of widespread pigmentation. Quincke (2) in 1877 observed with anæmia a deposition of iron in various organs notably in the liver and spleen and in one instance found macroscopic pigmentation of the liver and pancreas as a result of the iron deposition. Tillmanns (3) described brown pigmentation of the liver, of the abdominal lymphatic glands, and in less degree of the spleen and pancreas, in a man who two months before death had sustained a fracture of the pelvis and, the observer thought, a contusion of the liver. Hindenlang (4) observed in association with morbus maculosus Werlhofii pigmentation of the glands of the body, particularly the liver, which was slightly cirrhotic, and the pancreas, and in these organs found a pigment corresponding morphologically and in situation to that which v. Recklinghausen subsequently described as hæmosiderin. The presence of an iron-free pigment was not mentioned, but, being much less conspicuous than the hæmosiderin, it may readily have been overlooked, the gastro-intestinal tract not having received histological examination. Hintze (6) has described six cases which he regards as examples of hæmochromatosis. Several of them had previously been mentioned by Lubarsch (7). Only three agree in detail with the description which v. Recklinghausen has given. Two are the cases of extreme intestinal pigmentation before mentioned and in a third, the case of an individual, who five years before death had undergone an operation for removal of a cyst of the pancreas, the deposition of iron-containing pigment was limited to the pancreas and neighboring lymphatic glands. The pancreas was the seat of a chronic interstitial inflammation with cyst formation and the pigment was present only in the fibrous tissue while the epithelial cells contained none. Although the intestine presented the variety of pigmentation described by Goebel the condition cannot be regarded as hæmochromatosis and was probably the result of local hæmorrhage in or about the diseased pancreas. Buss (8) has described a typical case of hæmochromatosis associated with cirrhosis of the liver and diabetes mellitus.

Letulle records two cases and Richardière one in which associated with hypertrophic cirrhosis, accompanied by very abundant hæmosiderin pigmentation, there was similar pigmentation of the pancreas and of other organs. In these cases pigmentation of the skin was apparently absent.

A condition, associated with hæmochromatosis by v. Recklinghausen, has been studied particularly by French writers. In 1882 Hanot nd Chauffard (11) described two cases of diabetes mellitus associated clinically with hypertrophic cirrhosis of the liver and bronze-like pigmentation of the skin. At the autopsy upon the first of these cases was found cirrhosis of the liver, characterized by the presence of wide bands of connective tissue. In the liver cells, as well as in the interlobular bands, were brown pigment granules in great quantities. In the second case, more carefully studied, the liver and pancreas presented a brown pigmentation and were the seat of advanced chronic interstitial inflammation, both parenchyma cells and interstitial tissue containing masses of pigment granules. The stomach and duodenum were of a bluish-black color and pigment in small granules was found below the serosa. Letulle (12) several years later reported two cases of a similar nature. In a second communication Hanot in conjunction with Schachmann (13) recorded a fifth case and reviewed those previously published. He believed that the observations previously made established the existence of a new form of cirrhosis, *cirrhose pigmentaire diabétique*, and of a new clinical condition, *diabète bronzé*.

The designation "diabète bronzé" has been in general accepted, but not without protest. Bronzing of the skin is not a constant phenomenon and has been found absent in one case of Letulle, in the case of Hanot and Schachmann and in a case recorded by Brault and Galliard (14).

In the recorded cases of so-called bronzed diabetes,* as far as they are reported with sufficient detail, the symptoms and pathological findings are very constant. Clinically, the picture is one of a rapidly fatal diabetes mellitus associated with cirrhosis of the liver, usually of the hypertrophic variety. Bronzing of the skin is as stated not constant, but has been present in the majority of the cases. At autopsy has been found a deep pigmentation of the liver and pancreas associated with cirrhosis and in cases carefully examined macroscopically

* The published cases of bronzed diabetes, numbering twenty-four, have been carefully collected by Anschütz, and certain data are presented in tabular form in his comprehensive review of the subject which has appeared in the *Deutsches Archiv für klinische Medicin*, 1899, lxii, p. 411, subsequent to the preparation of this paper. For this reason a table of cases prepared by myself has been omitted.

and histologically interstitial pancreatitis. An ochre-colored pigment giving the microchemical reactions of iron is present in the parenchyma cells of the liver, pancreas and other glands, in the muscle fibres of the heart, in the interstitial tissue of these organs and in the lymphatic glands.

The facts relating to the incidence of the disease are of interest. No recorded instance of hæmochromatosis or of bronzed diabetes has occurred in the female. The age of the individuals affected with bronzed diabetes has varied between 33 and 62 years, the greatest number occurring in the fourth and fifth decades. The cases of simple hæmochromatosis fall within these limits. It has been believed that the disease is more common in France than elsewhere and this is to a certain extent indicated by the fact that of twenty-four cases seventeen have been observed in that country. Simple hæmochromatosis has been, however, described more frequently by German writers.

The pathogenesis of the condition has been the subject of varied speculation. Hanot and Chauffard and subsequently Hanot and Schachmann have maintained that the primary etiological factor is the diabetes mellitus, that the diabetic alteration of the blood in conjunction with endarteritis, which they have found constant in their cases, causes a disturbance in the nutrition of the liver cells, an alteration of the pigment metabolism and a deposition of pigment within the cell body. The excess of pigment so formed is reabsorbed by the capillaries and diffused possibly in the form of emboli over the entire organism. Letulle (12) finding the same process of pigment deposition in other organs, for example the heart, that takes place in the liver, comes to the conclusion that the pigment is formed in the cells in which it is found from the hæmoglobin of the blood. He regards the diabetes as primary and thinks that degeneration of hæmoglobin wherever it exists occurs under the influence of the hyperglycæmia. Brault and Galliard (14) also give prominence to diabetes as the important factor in the production of pigmentation. The hæmoglobin of the diabetic is, they think, so altered that it is incapable of the normal transformation into bile pigment and is deposited in the liver

cell. Furthermore, it accumulates in the blood and wherever pigment is elaborated and utilized.

Hernandez (16) thinks that dissolution of hæmoglobin takes place under the influence of a general malady, diabetes, and pigment is deposited in the glandular cells altering their nutrition and finally causing their destruction. The pigment is not eliminated, but passes into the lymphatics, and causes in their walls an irritation, manifested by an increase of the connective tissue of the organ. Mossé (17) believes that under the influence of the hyperglycæmia pigment granules are formed within the blood-vessels at the expense of the altered or dissolved hæmoglobin. The pigment accumulation in the liver is in proportion to the specialization of the liver cells to a chromogenic function and to the large volume of blood which traverses the organ. The coexistence of hyperglycæmia and melanæmia contributes in very great part to the production of cirrhosis. De Massary and Potier (19) support the conception that a diabetic alteration of the blood is the essential etiological factor in the production of the pigmentation. Rendu and de Massary (20) advance the hypothesis that the pigment is deposited in the various cells of the body as the result of an abnormal action exerted by the cell upon the hæmoglobin of the blood, the alteration of metabolism being the manifestation of a general cachexia caused by diabetes associated with cirrhosis of the liver.

A second smaller group of writers think that the pigmentation is produced by a primary disease of the blood, that as a result of some fundamental cause there is an alteration of the blood and subsequent formation of pigment from the altered hæmoglobin. The relation of the concomitant diabetes and cirrhosis then remain to be explained. Buss suggests that the glycæmia may be the result of an incomplete oxidation of oxidizable carbon, resulting, in turn, from a diminished oxygen-carrying power of the altered hæmoglobin. P. Marie (18) thinks that there follows the action of some primitive cause a dissolution of the hæmoglobin, which is transformed by the protoplasm of various cells of the body into pigment, deposited in them. The pigment in turn causes a degeneration and destruction of the cells in which it is accumulated and consequently chronic interstitial inflam-

mation of various organs, notably the liver. The bronzed diabetes is neither clinically nor pathologically the classic diabetes, but is a distinct morbid entity, as is according to his belief pancreatic diabetes, and if, he says, it should be necessary to compare the condition with any other he would turn his attention to pancreatic diabetes. Acard (21), Dutournier (22), and Jeanselme (23) reiterate the conception of Marie and suggest that perhaps the diabetes is only an accessory phenomenon which appears with a certain degree of chronic interstitial pancreatitis. Anschütz has convinced himself that the diabetes finds its cause in chronic interstitial pancreatitis which like the cirrhosis of the liver, he believes, is a manifestation of an underlying condition.

The French writers who have described cases of "diabète bronzé" do not identify the pigmentation with the hæmochromatosis of v. Recklinghausen. In the very early cases no examination of the chemical nature of the pigment was made. Hernandez demonstrated that the brownish-yellow granules found by him in the epithelial cells of the liver, pancreas and kidney, in the muscle cells of the heart, in the connective tissue of these organs and in the lymphatic glands gave the microchemical reactions known to be characteristic of iron. In this and in subsequent cases the brownish-yellow pigment agrees in morphology and in location with the hæmosiderin of v. Recklinghausen. No mention is made by the majority of the French writers of an iron-free pigment present in smooth muscle cells and in certain connective-tissue cells. It is the absence of hæmofuscin alone which raises doubt as to the identity of the pigmentation of bronzed diabetes with hæmochromatosis. The hæmofuscin, occurring in fine pale-yellow granules within cells, is much less conspicuous than the more coarsely granular hæmosiderin, and may very readily be overlooked in specimens treated with reagents which demonstrate iron. Auscher and Lapicque (24) studied the pigment present in the case reported by Marie. In addition to widely distributed ochre-colored pigment which became black on treatment with ammonium sulphide, they found on the intestinal wall a second variety of pigment which was according to them of black color and dissolved in ammonium sulphide. Quantitative determination of the iron of the intestinal wall demonstrated

a small percentage while that of the liver was extremely large. The pigment observed by Auscher and Lapicque and subsequently found by Acard and Dutournier, though described as black in color, is apparently the iron-free hæmofuscin.

Buss, in an inaugural dissertation, as mentioned, reports a case of diabetes associated with cirrhosis of the liver and of the pancreas and with general hæmochromatosis. He finds the iron-containing and the iron-free pigment in locations corresponding to those mentioned by v. Recklinghausen. On the one hand there is no reason to doubt that the pigmentation in this case is identical with that of hæmochromatosis and on the other hand the case presents the clinical and pathological picture to which Hanot has given the name "diabète bronzé."

The case described by the writer holds a position intermediate between hæmochromatosis and the so-called bronzed diabetes. Associated with hæmochromatosis there is bronzing of the skin, cirrhosis of the liver of advanced grade, and chronic interstitial pancreatis. Diabetes was, however, not present. It is evident then that the generalized pigmentation of bronzed diabetes is the hæmochromatosis of v. Recklinghausen.

When considering the etiological factors concerned in the deposition of great quantities of pigment in the liver and other organs we direct our attention to the blood, since it cannot be doubted that this iron-containing material is derived more or less directly from the hæmoglobin. It is well known that in pernicious anæmia with active blood destruction there is a deposition of iron within the liver and other organs, but pigmentation of the character under consideration is at least in the great majority of cases not found. A considerable proportion of the cases, both of simple hæmochromatosis and of hæmochromatosis associated with diabetes, have been accompanied by conditions which involve active destruction of the red blood corpuscles. Hindenlang's case of general pigmentation, almost certainly one of hæmochromatosis, was associated with morbus maculosus Werlhofii. In four other cases purpuric eruptions have been observed. In several there have been local hæmorrhagic conditions as, for example, hæmorrhagic pericarditis or peritonitis in cases of Hintze. In the

case of Buss there was found at autopsy hæmorrhagic pleurisy and peritonitis and hæmorrhagic pachymeningitis. Other forms of local hæmorrhage have been noted. The conditions cited present considerable variety, and in many cases were late manifestations in the disease. In many cases, moreover, local hæmorrhages were not demonstrable. It is then unnecessary to assume that the hæmoglobin forming the pigment arises from the destruction of red corpuscles of extravasated blood. It seems possible that, associated with some primitive alteration of the blood, there is a tendency to local hæmorrhage, the hæmorrhages being merely secondary manifestations of the same disease of the blood which, associated with intravascular destruction of red corpuscles, precedes the pigment deposition.

In a case of Jeanselme examination of the blood demonstrated the presence of a moderate grade of anæmia, but otherwise no marked pathological change of the blood was noted. Coagulation was not retarded.

Attempts have been made to reproduce experimentally the pigmentation found in human beings. Two methods have been employed: (1) The conditions of local hæmorrhage are produced, for example, by injecting blood into the peritoneum. (2) Hæmoglobin is set free within the circulating blood by the use of toxic substances which cause destruction of the red-blood corpuscles. Auscher and Lapicque (25) and Meunier (26) have attempted by these means to reproduce the pigmentation of the internal organs found in bronzed diabetes. Auscher and Lapicque by injecting blood into the peritoneum caused accumulation of an iron-containing pigment in considerable quantity in the spleen but in very small quantity in the liver. In cases of hæmochromatosis, however, the liver is the prominent seat of pigment accumulation.

It has long been known that toluylendiamin causes a destruction of the red-blood corpuscles, large doses producing in dogs hæmoglobinuria. By the use of this substance numerous experimenters have caused the deposition of an iron-containing pigment in the liver and other organs. Meunier, attempting to reproduce in dogs the pigmentation of bronzed diabetes, succeeded by the repeated injection of

small doses in obtaining an iron-containing pigment in moderate amount in the liver, lymphatic glands, spleen, and bone marrow. The immense iron accumulation of hæmochromatosis, however, has not been reproduced so that experiments have thrown but little light upon the pathogenesis of the condition.

The origin of the second form of pigment described by v. Recklinghausen, hæmofuscin, has been discussed at length by several writers who have studied hæmochromatosis. The data at hand are, however, very meagre. If the hæmofuscin were derived from the hæmosiderin, the two would be found side by side in the same cell and v. Recklinghausen, therefore, thinks that the hæmofuscin is formed independently from the hæmoglobin or some soluble derivative present in the lymph. Lubarsch and Hintze believe the hæmofuscin to be elaborated from the hæmoglobin as the result of a specific activity of the smooth muscle cells. Lubarsch injected defibrinated blood between the serosa and muscularis of the intestine of rabbits and obtained in the smooth muscle cells yellow granules of an iron-free pigment, formed, he thinks, by the action of the cells upon the liberated hæmoglobin.

It has been pointed out that a pigment apparently identical with the hæmofuscin of v. Recklinghausen is present in adults in the smooth muscle cells of the intestine under conditions which may be regarded as physiological, and according to Goebel increases with advancing age. In the sixteen cases of well-marked intestinal pigmentation without hæmochromatosis studied by him this pigment was not limited to the muscle of the intestine, but was found sparsely distributed in the smooth muscle cells of the blood-vessels of the intestine, kidney, liver, and lung, in the smooth muscle of the bronchi, urinary bladder, and prostate. He observed similar pigment in the spindle-shaped cells of the splenic trabeculæ and in connective-tissue cells of the liver, submaxillary gland, and mesentery. It seems evident then that the hæmofuscin found abundantly with hæmochromatosis occurs under conditions which may probably be regarded as physiological. Undoubtedly this pigment may occur unaccompanied by a generalized deposition of the iron-containing hæmosiderin.

Certain other iron-free pigments existing in moderate quantity un-

der physiological conditions have been in the case herewith described found increased. The iron-free pigment present in the heart muscle is greater in amount than that found under normal conditions. The interstitial cells of the testicle, normally containing pigment in moderate quantity, are loaded with pigment granules which do not give the reaction of iron. The pigmentation of the skin which has given name to the disease is caused by the presence in the deepest cells of the Malpighian layer of an iron-free pigment apparently not differing from that found under normal conditions.

It seems probable then that the same conditions which cause the deposition of an iron-containing pigment in various cells of the body favor the chromogenic metabolism of those cells which under normal conditions are capable of forming pigment. It may be pointed out, moreover, that hæmofuscin is present in considerable quantity in the walls of the vessels of the liver, pancreas, and lymph glands which contain hæmosiderin in great quantity; while in the vessels of organs like the kidney and lungs, in which is deposited very little iron-containing pigment, it is almost absent.

As already indicated a majority of those who have studied cases of bronzed diabetes regard the diabetes as the essential etiological factor. V. Recklinghausen so explained his case of hæmochromatosis associated with diabetes. Without sufficient basis apparently an active blood destruction is assumed to be the result of the diabetic condition. In the ordinary form of diabetes, however, an accumulation of iron does not take place in the liver or other organs, as shown particularly in cases studied by Zaleski * and Kretz.† A case of diabetes associated with cirrhosis of the liver without pigmentation is recorded by Hanot and Schachmann. Similar cases in which pigmentation was not, at least, a conspicuous feature are mentioned by Hansemann and other writers upon diabetes. There is, moreover, as will be pointed out reason to believe that the diabetes is secondary to the hæmochromatosis.

* Virchow's *Archiv*, 1886, civ, 91.

† *Beitr. zur klin. Med. u. Chir.*, Hft. 15, Wien, 1896; *Centralbl. f. allg. Path. u. path. Anat.*, 1897, viii, 620.

In the cases of bronzed diabetes the two prominent features in addition to the generalized pigmentation are diabetes and cirrhosis of the liver, and it is to this second factor that a number of writers have directed their attention in attempting to explain the pigmentation. It is to be recalled, moreover, that cirrhosis of the liver, usually of a less intense grade, has been present in the cases of simple hæmochromatosis. In all instances of hæmochromatosis, both with and without diabetes, the greatest pigment accumulation has been in the liver, and consequently a disturbed chromogenic function of that organ has suggested itself. It is conceivable that an iron-containing substance is deposited in the liver cells as the result of their inability to accomplish the normal elaboration of bile pigments from the hæmoglobin of the blood. This altered metabolism may be the concomitant of the cirrhosis of the liver, then to be regarded as the primary etiological factor. If the pigment is formed in the liver, how does it reach other portions of the body? Is there evidence that the pigment is transported by the blood stream either as emboli free in the plasma or as granules enclosed within phagocytic cells? An occasional particle of pigment has been found in blood obtained by pricking the skin, but the objection has been urged that such particles may be derived from the pigmented cutaneous glands. The demonstration in sections of an occasional pigment granule in the lumen of vessels is by no means conclusive, since an impervious material may in cutting be carried into such position by the knife. The demonstration, as in the case described by the writer, of cells containing pigment granules within the lumen of vessels is in no greater degree proof that in this way pigment is disseminated from a single depot of formation. For it is not inconceivable that from soluble substances in the plasma pigment is elaborated in those cells in which it is found. Moreover, as has been pointed out, it is probable that a certain number of pigment-containing cells are endothelial plates desquamated before death as a consequence of the pigment accumulation or after death as a result of postmortem change.

A study of the process of pigment accumulation in widely separated organs throws more light upon the seat of its formation. The glands

I have studied most carefully are the liver, pancreas, adrenals and glands of Brunner. When the pigment is in moderate quantity, it is present as relatively fine round granules occupying that portion of the cell most distant from the capillaries, that is, in acinous glands the portion of the cell next the lumen. With greater accumulation the whole cell body contains pigment granules and finally almost the entire protoplasm is replaced by them. In cells so loaded with pigment the individual granules vary much, occurring as large round globules, often of greater size than a red-blood corpuscle. Changes may also be observed in the nucleus of the cell. It becomes smaller, its outline often becomes irregular, and it assumes a shrivelled appearance. In cells appearing to be a mere mass of granules and globules of pigment with careful focusing a very faintly staining vesicular nucleus is occasionally seen, while more frequently are found pigment masses without any trace of nucleus, but still maintaining the cell outline. Finally the cell outline is lost and a clump of pigment particles of very variable size lies free in the tissue. The same process, observed in the liver, takes place in the pancreas and adrenal gland and, indeed, in the muscle cells of the heart. It is therefore, improbable that the pigment they contain is transported as emboli or in phagocytic cells from some distant organ, as the liver. Much more probable is it that the same process is taking place simultaneously in all the organs concerned, that the pigment is elaborated by the cells in which it is found from iron-containing material dissolved in the surrounding lymph.

Although, as it appears, the liver is not the seat of formation of the pigment found in other organs, a disturbance of the chromogenic metabolism of its cells may be an important factor in causing the deposition of pigment elsewhere. It may be that iron-containing derivatives of the hæmoglobin are not eliminated in consequence of changes in the liver cells and so accumulate in the blood. The extreme pigmentation of the liver, greatly exceeding that of other organs, may hold to its chromogenic function some relation which our present knowledge of iron metabolism does not explain.

Doubtless, for the production of a condition of hæmochromatosis, some factor is necessary besides the mere disintegration of red cor-

puscles and setting free of hæmoglobin. Its absence in conditions associated with blood destruction sufficiently proves this point. Of a series of cases of cirrhosis of the liver studied by Kretz * in about one-half was found an accumulation of an iron-containing pigment resembling hæmosiderin. He comes to the conclusion that the toxic matter circulating in the blood causes a degeneration of the liver cells and at the same time acts injuriously upon the red corpuscles. It is the association of the two conditions which simultaneously produces cirrhosis and pigmentation. Possibly in hæmochromatosis the toxic material causing the blood destruction is of such a nature that it acts injuriously upon the cells of the liver and other organs so that they transform the soluble blood pigment reaching them into the insoluble hæmosiderin. Alcohol may, under certain conditions, have this action. Such speculations merely indicate the complexity of the conditions which underlie the generalized pigmentation.

Degenerate cells overloaded with pigment are in the case studied very abundant and can be readily demonstrated without prolonged search. We have thus an efficient factor for the production of a chronic interstitial inflammation. Newly formed fibrous tissue invades the lobule along the central vein and in places sends out fine radiating bands along the capillaries between the columns of liver cells. The fibrous tissue about the central vein and that between the lobules contain large quantities of pigment for the most part extracellular as a result of the degeneration of the cells in which it was formed. This pigment lies free in the tissue presumably transported along lymphatic channels. When it is massed in large quantity, there is usually evidence of active cell proliferation. In general the interlobular tissue is poor in cells, but in such areas proliferation may be indicated by the presence of small round cells. Two factors are, I believe, active in producing the scleroses: (1) pigmentary degeneration of the parenchyma cells; (2) irritation produced by the presence of the pigment in the interstitial tissue.

In the case I have described the pancreas is the organ next to the liver most conspicuously pigmented. Extreme pigmentation and con-

* Loc. cit.

sequent degeneration of the parenchyma cells can be readily seen. Fibrous septa deeply pigmented separate the lobules and in places strands of similar nature penetrate between the acini. The pancreas has been very superficially examined in many of the reported cases of bronzed diabetes, but in those in which a description of the microscopic appearance is given it is evident that chronic interstitial pancreatitis existed. The fact that the organ in the great majority of cases has not been found atrophic has probably, in certain instances, prevented the recognition at autopsy of interstitial inflammation. In but two cases apparently has it been found to be smaller than normal while it is frequently described as voluminous, enlarged or normal in size. In seven cases the weight is recorded; the mean of the figures given, varying between 95 and 195 grm., is 125.7 grm., about one-half greater than the normal weight of the organ. The average weight of the liver, recorded in thirteen cases of bronzed diabetes, is 2497 grm. and we must regard the condition present as a form of hypertrophic cirrhosis. The lesion of the pancreas differs from the more commonly observed form of chronic interstitial inflammation, accompanied by more or less atrophy of the organ and may be designated as hypertrophic pancreatitis. In my case without diabetes the pancreas weighed 170 grm., almost twice the normal weight, and was the seat of an interstitial inflammation of moderate intensity.

The etiological relationship of chronic interstitial inflammation of the pancreas to diabetes has been well established in recent years by Hansemann and others, though the exact character and degree of such inflammation necessary to the production of glycosuria has not been definitely determined. Finding, with bronzed diabetes, chronic interstitial pancreatitis constant as far as we are able to judge, it is hardly possible to doubt that the diabetic condition is pancreatic in origin and makes its appearance when the pancreatitis has reached a certain grade of intensity. In the majority of cases the symptoms of onset are those of diabetes, that is the individual is apparently in good health until the onset of glycosuria. In the three cases of hæmochromatosis without diabetes described by Hintze, diseases having no apparent relation to the hæmochromatosis caused death. In the present instance inter-

current typhoid fever caused the death of the individual before chronic interstitial pancreatitis had reached a sufficient grade of intensity to produce glycosuria and thus the hæmochromatosis was prevented from reaching its usual termination, pancreatic diabetes.

CONCLUSIONS.

(1) There exists a distinct morbid entity, hæmochromatosis, characterized by the widespread deposition of an iron-containing pigment in certain cells and an associated formation of iron-free pigments in a variety of localities in which pigment is found in moderate amount under physiological conditions.

(2) With the pigment accumulation there is degeneration and death of the containing cells and consequent interstitial inflammation, notably of the liver and pancreas, which become the seat of inflammatory changes accompanied by hypertrophy of the organ.

(3) When chronic interstitial pancreatitis has reached a certain grade of intensity diabetes ensues and is the terminal event in the disease.

I desire to express my obligation to Dr. Welch and Dr. Flexner for the interest they have shown in the foregoing case and for the assistance they have given me in its study.

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THE ACTION OF HEPATIC, RENAL AND OTHER CELLS ON PHENOL AND INDOL, UNDER NORMAL AND PATHOLOGICAL CONDITIONS.*

By C. A. HERTER, M. D., and A. J. WAKEMAN, Ph. D.

It is the object of this communication to give the results of experimental observations on the behavior of various animal cells toward certain substances belonging to the aromatic type, notably phenol and indol. The inquiry was prompted by the interest which attaches to a study of the natural defenses of the organism against various kinds of damage through chemical agencies. Prominent among the problems that arise in connection with such an inquiry are, first, the determination of the seat of the defensive action of the organism, whether chiefly in the blood or in the cells; second, the relative activity of the different kinds of cells in the neutralization of the toxic properties of the particular chemical agents employed; and third, the character of any chemical transformation that may take place, when these substances are acted upon by living cells.

Indol and phenol were selected for use in the inquiry for two reasons. First, these substances are normal products of proteid cleavage in the intestine, under the influence of bacteria and of the proteolytic ferment of the pancreatic juice, and they are often formed in excessive amounts in the course of digestive derangements. Greater interest thus attaches to the fate of these substances in the organism than to that of wholly foreign poisons. Second, both indol and phenol are recognizable by means of delicate color reactions, and it becomes possible by means of the procedures to be described to make use of these color tests for the purpose of comparing the relative proportion of phenol and indol present in different solutions.

The Contact Method.—Two methods were pursued in our observations. In the first, the organs of healthy rabbits were quickly removed, after

* Read before the Association of American Physicians, May 4, 1899.

bleeding nearly to the point of death. After chopping the organs into fine bits, seven grammes of the pulp of the liver, kidney, brain muscle, and seven grammes of the blood were brought into contact with ten cubic centimetres of a weak solution of phenol and indol of known strength. The period during which these solutions were left in touch with the organ pulps varied in different experiments, two or three hours being the usual period. At the end of this time the mixture was subjected to distillation. The distillate was then tested for the presence of indol by the nitroso-indol test, and for phenol by means of Millon's reagent. By placing in columns equal heights of distillates in test tubes it becomes possible to arrange these in the order of the intensity of their color. Two or more members of the series sometimes give colors of equal intensity, or the difference may be so small that it is difficult to be certain of any difference. Usually, however, a good gradation of color can be obtained throughout the series. It is hardly necessary to state that the utmost care in technique is necessary in making these observations, by what we have come to call the "contact method." Under carefully controlled conditions it is possible to obtain reliable and consistent results.

It will be seen by reference to Tables I and II that the different organ pulps possess in different degrees the power of causing the disappearance of the phenol or indol with which they are brought in relation.

That all of them possess some activity, is seen by the fact that their distillates yield less color than a blank control of the solution, which has also been subjected to distillation. We may refer first to the results obtained with phenol and then to those with indol.

Order of Activity of Different Cells.—Of the twenty-seven observations on phenol, it is seen that the liver distillate gave the smallest amount of color in all twenty-seven cases, *i. e.*, in all instances the activity of the liver in disposing of phenol was greater than that of any of the remaining organs studied. In Table I in which the organs are arranged in series, according to the color reactions of their distillates, this fact is clearly brought out by the circumstance that all the livers are to be found in the right-hand column. The table shows further that the kidney occupies the next place to the liver as regards activity. Of the twenty-six cases in which the kidney was studied it comes into the column next that of the liver in eighteen. In the next

column the muscle occurs sixteen times. In the next column to the left the blood is found in twelve cases. In the next column to the left the brain is found eleven times. Finally, in the first column are seen all the control observations made upon blank solutions of phenol like those that we used in the contacts. In most instances it was possible to obtain a gradation of color. Where different tissues gave the same tint, this is indicated by the line drawn under the letters designating these tissues.

TABLE I.—EXPERIMENTS WITH PHENOL. CONTACT METHOD.

No. hours contact.						
3	Blank	Bn	Bd	M	K	L
3	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3½	"	Bn	Bd	M	K	L
4	"	Bd	M	K	Bn	L
3½	"	Bd	M	K	Bn	L
3½	"	Bn	Bd	M	K	L
3½	"	M	Bd	Bn	K	L
3	"	Bn	Bd	K	M	L
3	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	K	Bn	M	L
2	"	Bn	Bd	M	K	L
21	"	—	Bd	M	K	L
Few minutes	"	Bn	Bd	M	K	L
6	"	—	M	Bd	—	L
3	"	Bd	Bn	M	K	L
2	"	Bn	Bd	M	K	L
2	"	Bn	Bd	M	K	L
About 2 hours.	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	Bn	K	M	L
1	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	Bn	K	M	L

Bd=Blood, Bn=Brain, M=Muscle, K=Kidney, L=Liver.

Thus it is evident that in all instances the blood, brain, muscle, kidney and liver exerted some influence in transforming a certain amount of phenol so that it no longer responded to Millon's reagent. The results show very clearly that in point of activity the liver leads, with the kidney a close second and the muscle third. The blood and brain occupy the fourth and fifth places, although they are both less active than the muscle. The brain and blood are much alike in their behavior and appear to exert little action on the phenol. On the other hand, the action of the liver is often sufficient to yield only a very feeble color reaction with a solution of the strength employed.

Looking now at the results in the case of indol (Table II) it is seen by the table that here there is less uniformity than in the case of phenol. There are thirty observations in all, but in many of these a gradation of color through the series is impossible, so that many ties are noted in the table. Taking the liver first, it is seen that there are eleven cases in which it leads in activity. Twice it is tied with the kidney, once with the brain, twice with muscle and brain, twice with kidney and brain, and once with kidney, muscle and blood. In spite of these ties, however, it is clear that the liver leads the other organs in activity. But the kidney is a very close second and is found five times in the column indicating the greatest activity.

Without going into further detail, it may be stated that notwithstanding numerous irregularities, the table indicates that the order of activity of the cells acting on indol, is as follows: liver, kidney, muscle, brain and blood. Between the activity of brain and muscle there seems little difference.

In one respect these results differ from those obtained in the case of phenol,—the blood shows the smallest action of any member of the series, a degree of activity apparently less than in the case of phenol.

Isolated trials of the activity of the epithelium lining the small intestine of the rabbit indicate that these cells possess a high grade of effectiveness, comparable to that of liver cells. The cells of the spleen pulp are similarly but somewhat less active. Observations were also made with potato in order to ascertain whether the properties noted in animal cells pertain also to vegetable tissues. The results indicate a

moderate degree of activity on the part of potato in transforming phenol. Similar trials with egg albumen showed absolutely no action on the part of the proteid material. Experiments with gelatine gave entirely negative results.

TABLE II.—EXPERIMENTS WITH INDOL. CONTACT METHOD.

No. Hours Contact.						
2	Blank	Bn	Bd	M	K	L
3	"	Bd	M	K	Bn	L
3	"	Bd	Bn	M	K	L
2	"	Bn	Bd	M	K	L
4	"	Bd	Bn	M	K	L
20	"	Bd	L	Bn	M	K
20	"	Bd	M	Bn	K	L
4	"	Bd	M	Bn	L	K
4	"	Bd	Bn	M	L	K
4	"	Bd	Bn	M	L	K
4	"	M	Bd	Bn	K	L
4	"	Bd	M	K	Bn	L
3	"	Bn	Bd	M	K	L
3	"	Bn	Bd	M	K	L
4	"	Bn	Bd	M	K	L
20	"	Bd	L	Bn	M	K
20	"	Bd	M	Bn	K	L
4	"	Bd	M	Bn	L	K
4	"	Bd	Bn	M	L	K
4	"	Bd	Bn	M	L	K
4	"	Bd	M	Bn	K	L
4	"	Bd	M	K	Bn	L
3	"	Bd	M	K	Bn	L
3	"	Bn	Bd	K	L	M
2	"	Bd	Bn	K	L	M
3	"	Bd	Bn	K	M	L
3	"	Bd	M	Bn	K	L
—	"	Bd	Bn	M	K	L
20	"	L	Bd	Bn	K	M
3	"	Bn	Bd	K	M	L

Experiments were made to establish the influence of the time element in the contact procedure. In some instances the duration of the contact was twenty-four hours. The organ pulps subject to this long exposure showed no greater action than those exposed for only one hour. There is good evidence that the action is normally complete at the end of the hour and that the greater part of the transformation occurs in the first few minutes. Organ pulps protected against putrefactive change show very little diminution in activity even after the lapse of several days.

The Infusion Method.—The second method employed in the study of the action of the different organs on phenol and indol consists of making intravenous infusions of solutions of these substances. Usually the injections were made until nervous symptoms appeared. The animals were then killed promptly and definite weights of the liver, kidney, muscle, brain and blood were subjected to distillation. Color tests were then made on the distillates, so that the quantity of phenol and indol found in the different tissues might be roughly compared. In order to minimize the influence of an admixture of blood with the cell pulps the animals were bled to death.

It is, of course, evident that owing to the time that must elapse between the death of the animals and the beginning of distillation, some transformation must go on during the interval, and thus give rise to a source of error in the results. The operations were, however, carried on with the smallest delay and it does not seem probable that this source of error can seriously influence the results.

A review of the results shows that there is much confusion in the order of the color tints. Nevertheless a certain degree of order can be observed in the gradation of color. The muscle occurs in the right hand column in ten of eighteen observations made with phenol and in three further observations the muscle is tied with the blood or liver. There is thus no doubt that the muscle distillates in these experiments exhibit less color than those of any other organ. The interpretation of this result is not entirely clear. It may be due to the fact that less phenol has passed into the muscles from the blood or it may be due to the fact that the phenol entering the muscles is more rapidly trans-

formed than the phenol which goes elsewhere. The results of the contact experiments are perhaps of help in the interpretation of the behavior of the muscle. It will be recalled that these observations showed the muscles to be less active than the liver or kidney. Unless, therefore, we assume that the activity of the muscles is relatively less outside the body than when the blood is passing through them, it appears probable that the muscle takes up less of the phenol than do other organs. According to this view the position of the muscle in the injection experiments would be largely due to their slighter absorption of phenol from the blood.

As regards the position of the kidney, liver, brain and blood in the phenol injection experiments, it is difficult to establish a definite order in the color results. The liver and kidney in the average of results appear not to differ much. Both show more color than the muscles. This cannot be attributed, in the light of the contact experiments, to an inferior ability to transform the phenol, but is due rather to the greater storage of phenol in these organs during the injection. Finally the brain and blood show a still stronger coloration than the liver and kidney. This is not surprising, as the contact experiments clearly indicate that the blood and brain are comparatively inactive. In our injection experiments sufficient time has not elapsed for the withdrawal of all the phenol from the blood.

The observations relating to the infusion of indol resemble those with phenol in the irregularity of the results. Certain facts nevertheless stand out clearly. In the case of the indol as with the phenol injection, the muscle is generally found to contain less of the infused substance at the end of the infusion than any other tissue. Here again some difficulty arises in interpreting the results. Is the small content of indol due to small absorption or to active tranformation into some other substance? Referring to the experiments with the indol contacts, it will be recalled that the muscle exerts less energy in transforming indol than either the liver or the kidney. Unless, therefore, the absorption of indol were less than in the case of the kidney and liver the position of the muscle in the series could not be accounted for. It seems probable that the slighter absorption together with the

moderately active transforming power of muscle suffices to explain its behavior.

Another fact which deserves comment is the position of the liver in the series. In a very large proportion of cases, the liver yielded more color than any other tissue. This striking result can hardly be accounted for except on the ground that the liver is more active than any other tissue in removing indol from the blood. There certainly is no reason to think the liver less active than other tissues, if we can be guided by our contact experiments. Our inference is that the liver takes indol from the blood much more actively than it does phenol. The ability of the liver to dispose of the indol stored up at the end of the infusion is illustrated by the fact that if the animal be not killed until the lapse of twenty or thirty minutes, the amount of indol stored in the liver is no greater than that found in the other organs. This fact has been verified by numerous observations.

The following observation has reference to the exceptional behavior of the tissues in an individual animal. As an example of individual variation on the part of the cells it appears of sufficient importance to be mentioned here. A rabbit weighing 1900 grammes received an infusion of saturated indol solution at the rate employed in other observations. Before the injection was begun the animal seemed unusually nervous. After the injection was begun, spasm came on early and the pupils became very small. The irritative symptoms soon wore away but the animal continued restless. The total amount of the infusion was 28 c. c. of a saturated solution in water. The animal died half a minute after the close of the injection, and was bled from the heart. The striking peculiarity in the behavior of this animal during the infusion was that the nervous symptoms—spasm, contracted pupil and restlessness—came on much earlier than is usual. Another peculiarity of the animal was the readiness with which the muscle substance could be torn.

On studying the organs in reference to their content of indol, it was found that the kidneys, the liver and the muscle contained only the smallest traces of indol. The blood and the brain on the other hand contained a large amount of indol, that is, they yielded a strong color reaction. This behavior on the part of the various tissues is quite without parallel in our experience. It is evident that in this case the liver, kidney and muscle were unable to remove indol from the blood with the

usual promptitude. The brain on the other hand, took out a larger amount than is usual and one is tempted to connect the very pronounced nervous symptoms observed in this animal with the relatively large quantity of indol found in the brain. It also seems likely that the inability of the liver, kidney and muscle to remove the poison from the blood was dependent on some constitutional peculiarity.

Nature of the Action Exerted by the Cells.—In the course of the preceding pages reference has repeatedly been made to the activity of the liver, kidney, muscles, etc., in effecting the transformation of phenol and indol. What is the nature of this transformation which leads to the disappearance of a portion of the phenol and indol brought into relation with the living cells? This question, which has a high degree of biological interest, cannot at present be satisfactorily answered. There are, however, certain facts relating to the subject which may be briefly considered here.

In the first place, it is clear, from the observations cited, that the processes by which phenol and indol are altered, are carried on much more actively in the cells of the liver and kidney, and probably also in those of the muscle, than in the blood. The property of effecting these changes is unquestionably inherent in the cells themselves and the transformation in the organism must occur in the blood in comparatively slight degree. This fact is in accord with the teaching of modern physiology in reference to the seat of the oxidizing changes that occur within the body during life.

In recent years several investigators have devoted attention to the nature of the oxidizing processes that are carried on in the cells. The methods and results of certain of these observers have some resemblance to the methods and results that have been referred to in this paper.

Jaquet * appears to have been the first to make observations as to the occurrence of oxidating processes in cells that have been removed from the body. He found that in lungs cut out of the body, one gramme of benzyl alcohol added to blood circulating experimentally through the organs was oxidized and yielded 185 grm. of benzoic acid. Jaquet found

* Ueber die Bedingungen der Oxydationsvorgänge in den Geweben. *Arch. f. exp. Path. u. Pharm.*, 1892, xxix, p. 386.

that the oxidation was as complete if normal salt solution, instead of blood, was employed as the circulating fluid. Moreover he found that the tissues of the horse (kidney and muscle) were active even after immersion in 80 per cent alcohol for 14 days, and that an extract of fresh, or alcohol-hardened, tissues in salt solution possessed good oxidizing powers. These, however, were lost by boiling.

Jamagiwa,* a pupil of Salkowski, soon confirmed the results of Jaquet. He found that different tissues were active in the following order of decrease: (1) spleen, (2) liver, (3) kidney, (4) pancreas, and (5) muscle. Then W. Spitzer † found that extirpated tissues in general have the power of inducing certain oxidative syntheses, for example, the synthesis of naphthol and paraphenyl diamine to form an indophenol.

In recent papers W. Spitzer † attributes the oxidizing action of the cell to its nucleoproteid. He finds that certain constituents of the proteid molecule, such as histon, also possess the oxidizing power, and reaches the conclusion that the presence of iron is essential to these activities.

Salkowski ‡ in his more recent work concludes that the ferment whose action is noted in the case of cells removed from the body cannot be identical with the oxidizing ferment of living cells because they do not oxidize certain substances which are readily converted in the living body. This is true, for example, of the oxidation of phenylpropionic acid to benzoic acid.

Such evidence as we now possess certainly indicates that the oxidative changes carried on by cells removed from the body have reference to substances that are readily oxidized. Thus the conversion of benzyl alcohol to benzoic acid, of salicylaldehyde to salicylic acid, of arsenious to arsenic acid, of benzol to phenol, of formic aldehyde to formic acid, and of methyl to formic acid, are all examples of comparatively readily induced oxidations. We are as yet unjustified in holding that such changes go on with equal vigor in cells outside the body, and in the fully living cells. Similarly, with reference to syntheses,

* Ueber das Oxydationsferment der Gewebe, *Centralbl. f. d. med. Wissenschaften*, 1894, xxxii, p. 913.

† Die zuckerzerstörende Kraft des Blutes und der Gewebe, Pflüger's *Archiv*, 1895, lx, p. 303. Die Bedeutung gewisser Nucleoproteide für die oxydative Leistung der Zelle, *Ibid.*, 1897, lxvii, p. 615.

‡ Zur Kenntniss des Oxydationsferments der Gewebe, Virchow's *Archiv*, 1897, cxlvii, p. 1.

there are certainly some which the extirpated cells cannot perform. Spitzer was unable to confirm the statement that the synthesis of urea from ammonium salts can be made to occur outside the body. Our work with indol indicates that the synthesis of indoxyl potassium sulphate cannot be accomplished by extirpated cells. It also seems improbable that the dead cells convert phenol into phenol-sulphuric acid. Yet we know that these syntheses are performed in the organism, probably by the very cells which fail to effect them outside the body. It is natural to ask whether the known oxidative activities of the dying cells, to which reference has already been made, are the same activities that lead to the disappearance of phenol and indol during contact experiments.

The order observed by Spitzer, Salkowski and others in the activity of the different cells outside the body agrees closely with the order observed in our contact experiments with phenol and indol. This fact suggested that the changes observed in the case of phenol are in the nature of an oxidative transformation. The oxidation of phenol leads to the formation of pyrocatechin and hydroquinone, substances which are met with in the urine under certain conditions of disordered metabolism. With a view to determining experimentally whether such a transformation goes on in the case of phenol when this is brought into contact with fresh cells, ten kilos of ox's liver were reduced to a fine pulp and brought into contact with a solution of phenol and subjected to the contact procedure which has already been described. After a sufficiently long period of contact an endeavor was made to isolate any pyrocatechin or hydroquinone which might have been formed during the period of contact. It was found impossible to recover either of these substances. With the method employed it would have been possible to recover these bodies had they been present in even smaller quantity than might be expected from the action of so large a bulk of liver cells upon phenol.* The negative results obtained indicate that the disappearance of phenol which occurs after contact with liver tissue does not depend upon a process of oxidation into dihydroxy-benzene. This conclusion is supported

* The method used by E. Baumann. See Hoppe-Seyler's Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse, Berlin, 1893, p. 160.

by the fact that phenol introduced into the circulation leaves the body ordinarily as phenol sulphuric acid and not as hydroquinone or pyrocatechin.

In the case of indol it is thought that the first transformation that occurs in the organism is a process of oxidation into the indoxyl radical. It is possible that this oxidation occurs in the liver but this is by no means certain. If this be the case the action of the cells is perhaps comparable to the oxidative processes that have been studied by Spitzer and others. In what follows no further reference will be made to the fate of indol when acted upon by living cells. Attention will be confined to the discussion of the action of the cells upon phenol, an activity which apparently belongs in another category from that displayed toward indol.

The view held by Spitzer and by Salkowski that the oxidation which occurs through the action of cells is dependent on the presence of a ferment, raises the question whether such an agency will explain the behavior of cells toward phenol. The following facts bear directly upon this problem: Seven grammes of liver reduced to a pulp were brought into seventy-five cubic centimetres of absolute alcohol for one hour. The usual amount of phenol was then added to the liver pulp, and after a time subjected to distillation. The activity of the liver was slightly, but only slightly, impaired. In another experiment the usual amount of liver was boiled in fifty cc. of distilled water, and then brought into relation with phenol. Only the slightest falling off was noted in the action of the liver. In another case the liver was exposed to hot air, the temperature of the oven being gradually brought to 170° and kept there for twenty minutes. On exposure to the action of phenol, the liver showed no appreciable reduction in activity. Again, seven grammes of the liver were boiled with water, filtered hot, boiled again with water and filtered, and then boiled a third time and filtered. The pulp remaining on the filters showed itself capable of acting upon phenol with only a slight diminution in power. A test of the watery extract of the liver showed that it exerted only a very slight influence on phenol. Other trials made by bringing ten cc. of bichloride of mercury into relation with seven grammes of liver

showed that this procedure only slightly diminishes the activity of the cells.

Similar observations made with a 50 per cent solution of concentrated sulphuric acid yielded the same results. A 10 per cent solution of nitrate of silver was entirely without effect. In the case of the experiments with bichloride of mercury, sulphuric acid and nitrate of silver, the time of exposure of the liver cells was one hour.

In view of the facts just cited it appears in the highest degree improbable than any considerable ferment action can be attributed to the liver cells in connection with their behavior toward phenol. Another consideration which harmonizes with this conclusion is the fact that it is possible to completely exhaust the activity of the liver cells by repeated exposure to the action of phenol.

Although no wholly satisfactory explanation can be advanced as to the nature of the phenomenon under discussion, one is led to speculate whether we have not to deal with some loose combination of the phenol molecules with the molecules of the cell substance. That the combination between the cells and phenol is a loose one, is suggested by the fact that phenol absorbed from the intestine or injected into the circulation, reappears in the urine as phenol sulphuric acid.* It would also appear that the protoplasm of the cell suffers no damage when exposed to the action of phenol within physiological limits. The product formed by this combination of phenol with the cell substance may perhaps be likened to some of the numerous addition products known to chemistry.

The belief that the combination of phenol with the living protoplasm of the liver cells is a loose one, harmonizes well with the rapid elimination of phenol following the introduction of this substance into the circulation. It is obvious that it would be advantageous to the

* The phenol which enters the organism leaves the body almost quantitatively in the form of phenol, as is well shown by the following experiment: Into a large rabbit .080 grammes of phenol in watery solution were injected intravenously. The urine was collected for twenty-four hours and the phenol contained in the urine was determined by means of the iodine and sodium thiosulphate method. The quantity of phenol recovered was .0817 grms. It is possible that a few milligrammes of this phenol were derived from the intestine of the animal. The observation indicates, however, that the greater part of the phenol introduced was recovered.

organism for phenol to be temporarily held by the hepatic cells while the neutralization of the poison through combination with sulphuric acid is taking place. The manner in which such an arrangement would protect the nervous system, which is highly sensitive to the action of phenol, is sufficiently evident.

One other fact deserves mention in this connection. This has reference to the hypothesis advanced by O. Loew* that phenol acts upon living protoplasm by entering into combination with the labile groups which it contains, and, especially, groups possessing an aldehyde structure. With a view to testing this hypothesis the liver cells were subjected to the action of solutions of hydroxylamine, which enters readily into combination with aldehydes. Liver pulps treated in this way showed themselves to be as active or nearly as active in the conversion of phenol as normal livers.

In conclusion the opinion may be ventured that whatever may ultimately be learned of the nature of the phenomenon under discussion, it is likely that the capacity of cells to act upon phenol is only one expression of a function that can be exerted upon numerous allied aromatic substances, and it is even possible that a similar action may be exerted upon some non-aromatic bodies.

Action of Hepatic Cells under Pathological Conditions.—As already mentioned the degree of action exerted by the liver cells upon phenol and indol is fairly uniform. This fact suggested the possibility of recognizing alterations in the capacity of the liver cells to transform these substances under pathological conditions. It was, therefore, determined to make a series of studies of the liver cells of animals which had been subjected to different pathological influences. These observations include a study of the effects of prolonged anaesthesia, of poisoning by alcohol, ammonium chromate, morphine, etc., and of staphylococcus infection.

It is clear that it is a matter of considerable theoretical interest to know whether a definite impairment of functional activity in the cells of the liver and kidney is bound up with pathological influences like those just named. Efforts were, therefore, made to reach a decision

* Ein natürliches System der Gift-Wirkungen, München, 1893, p. 48.

upon this question although the difficulties attending these efforts were found to be somewhat discouraging.

The chief difficulty arises from the fact that the differences between the action of the normal and the pathological tissues are not great. This is what might have been expected in view of the resistance exerted by cells to the action of sulphuric acid, alcohol, etc. In order to establish the existence of such slight variations from the normal it was found necessary to exercise great caution in making comparisons between the distillates from the normal and the presumably pathological organs. It was soon found that the differences in the action of the kidneys and muscles were usually so slight in the pathological cases, as compared with the normal, that no inferences could usually be made. Only in the case of the liver—the organ in which action upon phenol and indol is the most energetic in conditions of health—was it possible to obtain satisfactorily results.

The distillates from the livers of pathological animals obtained through the "contact method" already described were compared not merely with individual distillates from the liver of normal animals, but also, in many instances, with mixtures of the distillates of the liver from many different animals. Great care was taken to make the conditions of the experiments the same as regards freshness of the organs, duration of the contact, quantity of distillates, amount of reagent added to give color, etc.

Anaesthesia by Ether and Chloroform.—The observations upon ether and chloroform included fifteen cases in which ether was used for anaesthesia and nine cases in which chloroform was employed. Of the fifteen cases of anaesthesia the liver cells were brought into contact with phenol in eleven, with indol in four. Of the nine cases of chloroform anaesthesia the livers were subjected to the action of phenol in four cases, to that of indol in five.

The rabbits subjected to etherization were kept under the influence of the anaesthetic for periods varying from five minutes to five hours in different cases. Although in four of the nine instances in which phenol contacts were made, the liver did not show the greatest activity of any member of the series, in the remaining cases the liver main-

tained its place as the most active organ. In most instances, however, the transformation of phenol was distinctly less than that effected by normal livers employed as controls. A similar difference was noted in the case of three of the four indol contact observations. In the fourth case where the animal had been under ether for two hours and a quarter the brain, blood, liver, kidney and muscle showed very slight differences in activity and the inference seems warranted that the activity of the liver, muscle and kidney are all below the normal.

The observations upon chloroform anaesthesia give even more definite results than those with ether. In two of the four cases where phenol was used (the animals being anaesthetized for three hours) the liver gave evidence of lessened activity. In the two remaining cases the liver, muscle and blood showed little or no difference in activity, a condition indicating that both liver and muscle were less active than normal. In four of the five observations with indol the liver was distinctly less active than the normal controls. In the fifth case the result was just the reverse.

Taking the results of these experiments with anaesthesia as a whole there can be no doubt that the prolonged action of chloroform and of ether depresses the activity of the liver in the conversion of phenol and indol. In exceptional instances it is not possible to detect this influence but the exceptions are so few that they do not affect the general result.

Poisoning by Alcohol.—Ten observations were made upon rabbits which had been subjected to the action of subcutaneous injections of 25 per cent alcohol. The periods of intoxication varied from a few hours to four days. The dose of alcohol was large in every instance and the animals were well under the influence of the poison. Thus in several cases the animal received 160 cc. of 25 per cent alcohol in three days and lay in a stupor most of the time.

Two of the ten observations were made with indol, eight with phenol. The periods of contact were varied from two to twenty hours in certain instances, the other conditions of the experiment being kept the same, but no differences in the results were detected which were referable to the differences in the length of contact. In a few cases

the activity of the liver was less than that of the normal controls, but more often no distinct falling off from the normal could be detected. Although we are not prepared to state that acute intoxication from alcohol is wholly devoid of effect upon the liver's action on phenol, our observations make it likely that there is either no influence of this kind or that it is very slight.

Poisoning by Ricin.—Thirteen trials were made to determine whether ricin poisoning exerts any influence on the ability of the liver to dispose of phenol and indol. It was thought probable that such an influence would be discernible as ricin has been shown to cause well-defined histological changes in the liver, kidney, etc.* The dose employed was unfortunately too large (.001 grm. to the kilo) and the duration of the poisoning was too short (less than thirty hours) to develop the degenerative lesions referred to. The results in the thirteen cases were negative.

Ammonium Chromate.—Observations were made upon four rabbits poisoned with ammonium chromate. The animals received two doses hypodermically of .025 ammonium chromate and were killed on the third day. The behavior of the liver cells did not positively differ from that of normal cells subjected to the same process of contact with phenol and indol previous to distillation.

Staphylococcus Infection.—The influence of staphylococcus infection was studied in two cases. The rabbits received five cc. each of a culture of *Staphylococcus pyogenes aureus* grown two days in bouillon. The organism was obtained from a patient with follicular tonsilitis. The animals were permitted to live one week, during which time they lost considerable weight. They were then killed by bleeding and the tissues subjected to the usual procedure. In both cases the differences in the activity of the brain, blood, muscle, kidney and liver were less than is usual in normal series. The distillate from the livers in the two cases was more colored than is usual in the case of normal livers.

These observations appear to indicate that in staphylococcus septi-

* Flexner, The Histological Changes Produced by Ricin and Abrin Intoxications, *Journal of Experimental Medicine*, 1897, ii, p. 197.

caemia there is some impairment of the activity of the liver and kidney, but they can hardly be looked upon as conclusive.

Morphine Poisoning.—Eight observations were made upon rabbits under the influence of morphine. The animals received from five to eight grains of sulphate of morphine. Some of the animals lived only a few hours, others lived as long as two days. In four instances the activity of the liver as compared with that of normal controls was apparently somewhat lessened, but the deviation from the normal state was very slight. In every case except one the muscle showed greater capacity for transforming phenol than did the normal muscle. In several instances the difference was considerable as indicated by the color reaction. In several instances the kidney also showed an apparently increased activity.

Double Nephrectomy.—In five instances a double nephrectomy was performed with a view to seeing whether the removal of the kidneys is instrumental in impairing the activity of the liver. The animals were killed in from twenty-two to twenty-four hours. In all cases the liver showed a slight falling off in activity as compared with normal controls. The muscle and kidney, as in the case of morphine poisoning, showed in all cases somewhat greater capacity than usual but the differences were slight.

Infusion of One per cent Acetic acid Solution.—With a view to learning whether a reduction in the alkalinity of the blood has any influence upon the liver, a 1 per cent solution of acetic acid in a 1 per cent solution of sodium chloride was infused intravenously at the rate of 5 cc. a minute. The animal, a medium sized dog, went into coma before the completion of the infusion, which consisted of 130 cc. The animal excreted no urine during the period of injection. It was found that the activities of the liver, brain and muscle were about the same and distinctly inferior to the action of the kidney. A similar relationship has not been observed in normal dogs and it seems probable that the infusion of acid distinctly reduced the activity of the liver.

A similar observation with acetic acid, conducted on a large rabbit, gave a result comparable to that just noted in the case of the dog.

Benzoic Aldehyde Poisoning.—In these instances a saturated solution of benzoic aldehyde in water (1 part to 30) was infused into the venous circulation at the rate of 5 cc. per minute, until the animals died after developing symptoms of poisoning. The quantity of the solution infused varied from 80 to 210 cc. in the different cases. The livers from these rabbits showed some falling off in relation to their action on phenol. It was found, however, that the benzoic aldehyde employed in the experiments gave a slight reaction with Millon's reagent, and it is therefore possible, though not probable, that the abnormal behavior of the liver depends on the presence of phenol in the benzaldehyde in addition to the phenol added in the course of the contact procedure. It is to be regretted that this source of error exists, as it would be interesting to know whether the exhaustion of the oxidative activity of the liver upon a readily oxidizable substance like benzaldehyde necessarily causes impairment of the ability to transform or bind phenol.

Infusion of Urea.—Infusions of urea were made in several instances with a view to overworking the kidneys and observing whether such overwork causes any impairment in the activity of the kidney in converting phenol. The results obtained were wholly negative.

In addition to the various experimental observations recorded above, a number of trials were made upon fatty and cirrhotic livers from human subjects. Owing to the delay incidental in obtaining human material after death it was impossible to obtain satisfactory results. It may be stated, however, that no marked deviations from the normal capacity of the liver to convert phenol were observed in organs the seat of the most advanced structural alterations.

It is probably safe to conclude that no pathological conditions which can be induced in the liver during life are capable of destroying, or even of greatly impairing, the activity of its cells in effecting the conversion of phenol. What is true of phenol in this connection is likely to hold good of indol. This view is supported by the few observations which have been made on indol.

The fact that this converting function of the liver is not greatly impaired in disease might perhaps have been predicted from what has

already been said of the remarkable functional resistance which the liver exhibits to the influence of injurious agencies outside the body. This preservation of function in disease is perhaps merely another expression of the fundamental biological properties which we have seen manifested by cells extirpated from the body. It is only reasonable to think that these properties are closely connected with the ability of the body cells to screen the organism and especially the nervous system from certain poisons. If this be so, the phenomena referred to in these pages deserve further study from pathologists.

ON THE INTERPRETATION OF PULSE-TRACINGS.

By ARTHUR R. CUSHNY.

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PLATES XVI AND XVII.

The physiology of the mammalian heart has advanced with such rapid strides of late years, that clinical observers have apparently had difficulty in keeping pace with it, and but little attempt has been made to adapt the theories of the experimental investigators to the observations made on the diseased heart. It is true that the explanation of the undulations on the pulse-tracing and of their modifications in pathological conditions has engaged the attention of a considerable number of observers, but until quite recently the divergences of the cardiac rhythm from the normal seem to have aroused little interest, and no attempt was made to explain the origin of the irregularities of the pulse. In the following pages I have endeavored to fill in one hiatus existing between clinical observation and physiological experiment.

The ventricular systole which causes the radial pulsation is, in the normal heart, induced by an impulse descending from the auricle through communicating fibres which are generally held to be formed of muscular tissue; the normal ventricle never contracts unless it is excited by the arrival of such an impulse from above. It is obvious that an irregularity in the ventricle (and consequently in the pulse-tracing) may arise (1) from the ventricle failing to respond normally to this impulse or from its contracting independently of it or (2) from the auricle failing to emit an impulse at the ordinary interval. Yet it is only of recent years that the work of Krehl and Romberg* has led to the general recognition that an irregularity of the pulse may be due to disorder of the higher parts of the heart, which determine the normal rhythm. Nor has the importance of the auricle as a factor in

Arch. f. exp. Path. u. Pharm., 1892, xxx, p. 49.

ventricular irregularity been appreciated apparently by pathologists as yet, for, with the exception of Radasewsky's* paper, I have been unable to find any accounts of examination of the auricle in cases of irregular pulse.

My attention was drawn to the subject by the results of a series of experiments performed some years ago along with Matthews and during the last year I have through the kindness of Dr. Dock and his staff had the opportunity of examining a considerable number of sphygmographic tracings taken in cases of irregularity of the pulse. I propose in the following pages to attempt to interpret these clinical observations in terms of our experimental results.

The object of our experiments, which were performed on dogs, was to ascertain the effects of a single stimulus applied to the heart. It is unnecessary to revert to most of our conclusions, which bear rather on physiological than on practical points, and which have been published elsewhere.† Let it suffice to state that the movements of the auricle and ventricle were recorded separately by a system of levers, and that in a number of instances a sphygmographic tracing was taken simultaneously from the carotid artery. When a single electric shock was passed through the ventricle at any time except in the refractory period, it was followed by a premature contraction of that chamber (Fig. 1, p. 329). In many instances this contraction expelled sufficient blood to cause an undulation in the sphygmographic tracing, this undulation being lower than that caused by a normal pulse and occurring at a shorter interval than usual after the last ordinary elevation. But as a general rule the contraction of the ventricle (premature contraction) was too feeble to cause a pulsation of the artery. In Fig. 1, a premature systole (*c*) of the ventricle was induced by an electric shock which reached that chamber at a point indicated by the crosslines × in the ventricular tracing. It was succeeded by relaxation, and the ventricle then remained quiescent until it was aroused to activity (*d*) by an impulse descending from the auricle. The auricular rhythm was unaffected by the irregularity of the ventricle. If the course of each impulse be followed from the auricle to the ventricle and finally to

* *Ztschr. f. kl. Med.*, 1895, xxvii, p. 381.

† *Journal of Physiology*, xxi, p. 213.

the pulse, it is found that A is followed by a and then by a, B by b and then by b. But C beginning in the auricle has no effect on the ventricle, which it reaches during the premature systole c, *i. e.* during a refractory period. D is followed by d, however, and later by d and the normal sequence is then reinstated. The interval between the normal pulsations b and d or between the ventricular systoles b and d is determined by that between the auricular contraction B and D. And as the auricular rhythm is perfectly regular throughout, the interval B—D is twice that of B—C or twice A—B or D—E. That

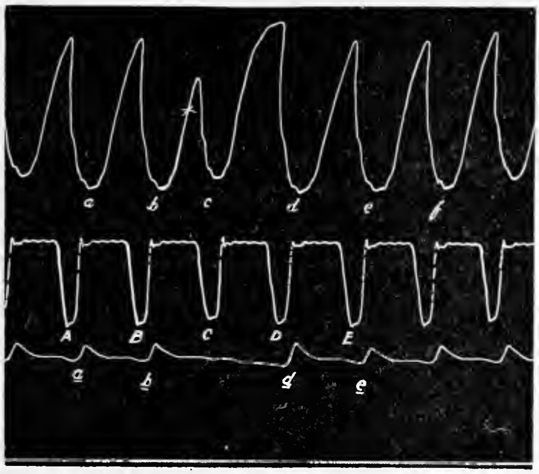


FIG. 1.

In Figs. 1 and 2 the upper tracing was drawn by the ventricle, the next by the auricle, the third by a sphygmograph (Frey's) attached to the carotid artery, and the fourth by a tuning-fork swinging 50 times per second. During systole the auricular and ventricular levers made a stroke downwards. During diastole they rose again. For further explanation see text.

is, the interval between the two normal pulsations of the artery, or the duration of the intermission, is equal to twice the usual interval between two pulsations (pulse-interval).

A little consideration will show that as long as the auricle is beating regularly, an intermission which is due to ventricular failure must always be equal to twice the ordinary interval. For example, had there been no premature contraction (c) in Fig. 1, but had the ven-

tricle relaxed and remained quiescent until it was aroused by the impulse *D*, the length of the interval would have been the same. So that a general law may be formulated, that *an intermission which is due to ventricular disorder and during which the auricle continues to contract regularly must always be equal in length to twice the ordinary pulse-interval.*

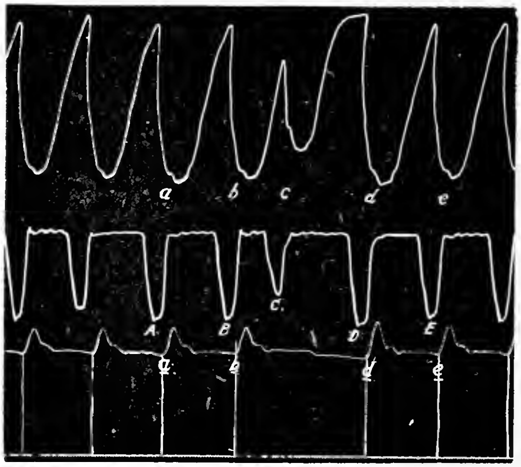


FIG. 2.

For explanation see Fig. 1 and the text.

When an electrical shock is passed through the auricle (Fig. 2), a premature systole is caused in that chamber exactly as in the ventricle, but it does not remain limited to the auricle but is transmitted to the ventricle, which contracts prematurely as if it had received a direct stimulus; a weak premature pulse is sometimes seen in the artery, but here again the blood expelled is generally too small in amount to cause any appreciable movement and the tracing displays a complete intermission. In Fig. 2, *A* gives rise to *a* and *a*, *B* to *b* and $\underline{b}$ and *C* to *c*, which is not followed by a distinct movement in the artery. The next normal auricular systole, *D*, is followed by *d* and *d* and *E* by *e* and $\underline{e}$. The only difference from Fig. 1 apparent at first sight is the irregularity of the auricle, and as this is not visible in the sphygmographic tracing, it might be supposed that an irregularity of auricular

origin is followed by the same changes in the pulse as one arising from ventricular disorder. When the sphygmographic tracing is subjected to exact measurement, however, a difference is observed in it; the intermission is shorter than twice the ordinary pulse-interval. For example, if the intervals *a—b*, *b—d*, and *d—e* are measured, it will be found that *a—b* and *d—e* are each equal to $\frac{27}{50}$ sec. If the intermission *b—d* were twice the normal interval, it would last $\frac{54}{50}$ sec., but in reality it is only $\frac{49}{50}$ sec. in duration. The explanation of this point is simple—the auricle itself gives rise to the rhythm of the heart. The impulse leading to the contraction *D* is not derived from any higher rhythmical organ, but arises from the auricle itself. When the auricle has collected enough energy or irritability to cause a contraction, the systole follows, this chamber not waiting as the ventricle does for some impulse from without. The exact length of the intermission in these stimulation experiments varies with the point in the auricular cycle at which the electric shock was given, but it is unnecessary to enter on this here. The important fact is that in these experiments the intermission was always shorter than twice the ordinary pulse-interval. Consideration will show that here again a general rule may be formulated, that the intermissions due to auricular disorder are not necessarily equal in length to twice the ordinary pulse-interval. For example, the auricle may stand still for any interval of time and then resume its contractions. It is not necessary that it should contract at the exact time at which it would have done so normally, any more than it is necessary that if the breath be held for some time, the next inspiration will follow at any definite moment. When the probabilities of the case are considered, it will be seen that the chances of the auricle resuming its contractions at the end of a double pulse-interval are small. The irritability of this chamber increases very rapidly when it is quiescent and in most cases must reach the point at which a contraction is elicited sooner than after two intervals.

These considerations appear to be sufficient basis for the general statement, that *intermissions of the pulse which are due to ventricular disorder must be equal in length to two normal intervals, while those*

that are of auricular origin may be of this length, but in the great majority of cases are not of this duration but shorter. Conversely, intermissions of the pulse which are equal to two ordinary intervals, are in all probability ventricular in origin; those which are shorter are certainly not ventricular and are therefore due to auricular derangement.

A few examples of the duration of the intermissions in the dog's pulse under stimulation of the ventricle and auricle may serve to elucidate this question (Table 1, p. 343). It may be stated that these do not by any means exhaust the numbers at my disposal, for I have made a large series of experiments on the subject and have measured some hundreds of intermissions, all of which gave the same result without exception. I have selected some of the measurements of only two of these experiments, however, which are sufficient to give an idea of the extent of the variation in the length of the intermissions. When the stimulus resulted in a premature arterial pulse, the intervals before and after it are enclosed in brackets, in order to allow of a comparison with those in which there was a complete intermission. In column A, the duration of the normal interval is given in hundredths of a second; in column B, the part of the heart stimulated; in column C, the duration of the intermission in hundredths of a second; in D, the extent by which this fell short of two complete intervals ($2A—C$); and in E, the fraction of a pulse-interval represented by this deficiency $\frac{D}{A}$. In the last column the averages of, and the extremes of, E are given approximately.

In Table 1, it will be observed that when the intermission is due to ventricular stimulation, it is equal to a double interval, the greatest deviation from this amounting to only $\frac{1}{54}$ and $\frac{1}{40}$ of the normal pulse-intervals, numbers which may well be considered within the limits of error. When the stimulus was applied to the auricle, on the other hand, the resulting intermission was invariably shorter than a double pause, the deficiency varying from $\frac{1}{13}$ to $\frac{2}{5}$ of a normal pulse-interval according to the exact position of the auricle when the stimulus reached it. It may be added that the deficiency differs in

different hearts, that in Experiment 2 being distinctly greater than in Experiment 1; *i. e.*, in the second experiment, the auricle seems to regain its irritability more rapidly than in the first, and this is probably to be correlated with the fact that the rhythm is faster in the second.

When one attempts to apply the principles deduced from these animal experiments to analysis of clinical sphygmographic tracings, one is met by the difficulty that in some of these the pulse is so irregular that no normal interval can be ascertained with certainty. These may be passed over at present and only those tracings will be considered in which the rhythm is regular on the whole, but in which intermissions or other irregularities appear at longer or shorter intervals. In these also there occur small differences in the pulse-interval, and even in some perfectly normal individuals a considerable variation has been shown to exist by von der Mühll.* These differences seldom amount to more than one or two hundredths of a second, however, so that they do not materially affect the results. In order to eliminate the error so far as possible, I have generally taken the average of three or four pulses preceding and following the irregularity and thus obtained an average interval, which was compared with that immediately preceding and immediately following the intermission. In this way certainty could be reached that the interval taken as a basis for the calculation really was that which would have occurred had no irregularity been present, or at any rate did not depart very far from this ideal interval.

The tracings were taken with Jaquet's sphygmochronograph, which is the only instrument available when such minute intervals as $\frac{1}{100}$ second have to be dealt with. In analysing the tracings I have used his curve-reader (Curvenanalysator), which was kindly put at my disposal by Professor Lombard. A number of tracings were taken in each case, and the irregularities were all carefully measured and compared, and an average taken of the extent to which they fell short of a double interval. Only in this way can any reliable results be attained, as not infrequently one individual intermission may appear to be of auricular origin, while when it is compared with the others of

* *Deutsches Arch. f. klin. Med.*, 1892, xlix, p. 348.

the series it is seen to be so nearly equal to two pulse-intervals that it falls into the group of ventricular intermissions.

In Table II (pp. 344-5), I have arranged a series of intermissions or irregularities (premature pulsations), which approach the type found to be due to ventricular stimulation in the dog; and in Table III (pp. 346-7) intermissions approximating those from stimulation of the dog's auricle. The columns correspond to those of Table I (p. 343) except that the second column is omitted.

In Table II, the average deviation from a double pulse-interval varies from zero up to $\frac{1}{20}$ of a pulse-interval. In regard to Case W, I had some doubt whether it should be placed in Table II or in Table III, and the number of tracings at my disposal was not sufficient to permit a certain interpretation. In Table III, the average intermission was $\frac{1}{5}$–$\frac{1}{3}$ of a pulse-interval shorter than two pulse-intervals.

When Tables II and III are compared with Table I, there is noted a very striking resemblance between the intermissions due to ventricular stimulation and Table II, and between those due to auricular stimulation and Table III. This resemblance at once suggests that the disorder in the hearts from which Table II was derived was ventricular, while the cases from which the tracings analysed in Table III were obtained, suffered from disease of the auricle. It may be added that the line of demarcation between the two sets of tracings is very sharply drawn. In Table II, the fractions in column *E* are all so small that they may be regarded as lying within the limits of error, while those in Table III, column *E*, are so large that no such explanation is possible. There were no intermediate cases except W (Table II), and even in this tracing the intermissions are very different from those of Table III. It may be added that these were not selected tracings, but were those obtained from 14 of the first 16 cases of irregularity which came under my observation. In the remaining cases the tracing was so irregular that no definite pulse-interval could be recognized, and they have therefore been omitted from the tables. None of the intervals in these tracings were double the others in length, however, so that there can be no doubt that the irregularity was due to auricular derangement. In all of these cases, then, a care-

ful examination enabled a diagnosis to be formed as to whether the irregularity was due to functional disorder of the auricle or of the ventricle.

The further question arises as to what is the nature of the intermission of the auricle or ventricle in these cases. The heart may fail to cause a pulse-wave, firstly, because the ventricle contains so little blood when it executes a premature contraction that the arterial pressure is not increased enough to move the sphygmograph, or, secondly, because the ventricle entirely fails to contract or contracts too weakly. A very important contribution to this subject has been made by Wenckebach* since I began my investigations; in fact this writer has anticipated many of the conclusions at which I had arrived independently.† He discusses almost exclusively the intermissions which are equal to two pulse-intervals and states that these are invariably due to premature idioventricular contractions. His grounds for this view are two-fold in nature: in the first place he was able in every case to make out by auscultation a double contraction sound in the beginning of the intermission, and in the second place a pulse-tracing which at one time shows a complete intermission very often shows immediately afterwards an intermission interrupted by a weak premature elevation. I have met this in most of my own cases, and there is no question that many, perhaps most, cases of ventricular intermission are due to this premature contraction (extrasystole, Wenckebach) so that they correspond in every feature with those obtained in my experiments on the dog, except that in man the ventricular stimulus arises spontaneously in the ventricle while in my experiments it was artificial. In a numbér of instances, however, I have observed an extrasystole in the dog without any electrical stimulus having been supplied, and in these cases the intermission was identical in every feature with that observed in the human pulse.

It may be questioned, however, whether this is the explanation in every case of ventricular intermission, as Wenckebach holds, for in one

* *Ztschr. f. klin. Med.*, 1899, xxxvi, p. 181.

† Among other points he noted that in many cases the intermission fell short of two pulse-intervals, and ascribes these to auricular disorder, basing this explanation on our experimental results already referred to.

of our patients the most careful stethoscopic examination failed to detect any systolic sound during the intermission. I am inclined to regard the intermission in this case as due to a complete failure of the ventricle to respond to the auricular impulse, or to the impulse having been blocked in the communicating fibres between the auricle and ventricle.

As regards the intermissions which are caused by auricular derangement, the cause is unquestionably a premature auricular contraction in a certain number of instances. This is demonstrated by the appearance of a distinct undulation in the pulse-tracing in some cases (Plate XVI, Fig. 8), while in others a number of complete intermissions are interrupted by one which shows this premature pulsation. These intermissions are therefore analogous to those observed in the dog on stimulation of the auricle, for here also the fall of the pulse-lever is sometimes unbroken (complete intermission), at other times interrupted by a slight undulation. In the clinical sphygmographic tracings the premature systole of the auricle is of course caused by some intracardiac stimulus, and I have therefore sought for descriptions of auricular disease in the literature of the heart, but have as yet found only one paper, viz.: that by Radasewsky,* in which the auricle was subjected to careful post-mortem examination with reference to this point. He states that of six hearts examined by him four presented more severe lesions in the auricle than in the ventricle and in these cases there was a history of marked irregularity of the pulse; in one in which the ventricle alone was diseased, and in another in which both auricle and ventricle were less extensively degenerated, no marked irregularity had been noted before death. In one of my cases in which the measurement of the pulse demonstrated clearly that the intermissions were of auricular origin (Table III, Case Sh), the autopsy showed an enormous dilatation of all the chambers; the auricular walls were thin and transparent and the muscular bundles formed a network separated by wide meshes composed of the serous membranes only.

Those cases in which the irregularity is due to an extrasystole arising in the auricle are indicative of some disorder in this chamber,

* *Ztschr. f. klin. Med.*, 1895, xxvii, p. 381.

which may prove to be of grave import, though my experience has been too limited to allow of any certain statement as to the value of this sign in prognosis. But every case of intermission in which the interval is shorter than two complete pulses does not necessarily indicate the presence of auricular disease. In fact this probably holds for only a small proportion of the cases in which it occurs; for these lapses are very often seen in perfectly healthy persons, and no line can be drawn separating them from the slight irregularities of the normal pulse. For example one of my tracings, taken from a healthy woman, shows a somewhat irregular rhythm, the pulse-interval ordinarily varying from 54 to 61 hundredths of a second, but occasionally an intermission of 90 to 100 hundredths of a second appears. These intermissions can be elicited with regularity by instructing the patient to take very deep inspirations, and are obviously not due to auricular disease but to excessive activity of the inhibitory centre. As is well-known, this centre is peculiarly sensitive in some individuals, and in this case it is capable of arresting the heart completely for a short time. The whole heart is arrested and the intermission is therefore of the auricular type. Exactly the same phenomenon can be elicited in animals by stimulating the pneumogastric nerve in the neck for a fraction of a second, as may be seen by comparing the sphygmogram from a healthy woman (Plate XVII, Fig. 13) with that from a dog whose vagus was stimulated electrically (Plate XVII, Fig. 14).

This inhibitory intermission is liable to be confused with the true auricular intermission, and in fact I am inclined to regard the irregularity in the first case in Table III as due, at any rate in some part, to inhibition. There are certain distinguishing features, however; in the first place the intermission is often followed by a second long interval, because the inhibition seldom lasts over only one beat; in the second place, the intermission is often, but not always, too short to arise from auricular irregularity. Thus the shortest intermission which I have observed in the dog's heart from stimulation lasted $1\frac{3}{5}$ pulse-interval, but the inhibitory intermission may be only $1\frac{1}{5}$ pulse-interval. In case of doubt the question could be determined by the pulse being rendered regular when the inhibitory terminations are

paralyzed by atropine, for this drug is without effect on the true auricular intermissions. Dehio* has observed arythmia disappear under this treatment in a number of instances.

The occurrence of this false auricular intermission suggested the question whether in some cases ventricular intermissions might not be due to inhibition, for it is well known to physiologists that stimulation of the pneumogastric nerve retards the passage of impulses from the auricle to the ventricle, and renders the latter less susceptible to stimulation. It is thus conceivable that some of the ventricular intermissions might be due to the impulse from the auricle finding the passage to the ventricle blocked or the ventricle incapable of responding to it owing to powerful inhibition; but there seems to be no reason to suppose that this occurs, for the rhythm of the heart would be reduced to 20-30 per minute or less by inhibition powerful enough to block the communicating fibres or to prevent the ventricle entirely from responding to a stimulus. It is very probable that in many instances intermissions of different kinds may occur in the same pulse-tracing. For example, both ventricle and auricle may contract prematurely at intervals, or an intermission due to a premature systole of the ventricle may alternate with one due to inhibitien. I have not been able to identify such a mixed tracing with certainty among those hitherto examined, but in one or two of them in which the irregularities were mainly of the auricular type an occasional intermission approached so nearly to the double pulse-interval, that it aroused the suspicion that the ventricle also was involved.

I have stated that an excessively irregular pulse indicates auricular rather than ventricular derangement, but this requires some qualifications. For example, Fig. 10 (Plate XVII) was obtained from a patient in whom ventricular intermissions occurred frequently, but who never presented any signs of auricular disorder. Yet the pulse-intervals in Fig. 10 are 48, 47, 48, 48, 49, 47, 41, 95, 50, 34, 60, 47, 47, 48, which would seem at first sight to indicate disorder of the rhythmic area rather than of the ventricle. The explanation appears to be that several ventricular premature systoles occurred in succession, that *a*, *b*

* *Deutsch. Arch. f. klin. Med.*, 1893, lii, p. 97.

(a rudimentary beat), *c* were all due to idioventricular contractions, while *d* was due to an auricular impulse and *e* was again idioventricular. Thus the intervals are $41 + 95 + 50 = 186 = 4 \times 47 - 2$; *i. e.*, while the ventricle was beating *a*, *b*, and *c*, the auricle was contracting regularly three times but in a slightly different rhythm. The fourth auricular contraction was propagated to the ventricle. A premature idioventricular pulsation followed this and the sixth auricular beat again reached the ventricle and induced *f*. That the auricular rhythm was regular is shown by the sixth ventricular contraction occurring at the exact point at which it would have fallen had there been no irregularity at all (*i. e.* $41 + 95 + 50 + 34 + 60 = 280 = 6 \times 47 - 2$) for the slight difference of $\frac{2}{100}$ of a second can be neglected. In several other instances a similar apparent irregularity can be reduced to several idioventricular beats following each other, as has been demonstrated by Wenckebach. These repeated premature systoles certainly present difficulties in analysis, but in cases in which the tracings indicate uniformly ventricular intermissions, it is of interest to attempt their solution as instances of ventricular derangement only. If the other sphygmograms in this case had not invariably indicated a perfectly regular auricular rhythm, I should have hesitated to interpret this particular irregularity as ventricular.

This repetition of the ventricular extrasystole suggests the question as to whether in some cases the pulse-rhythm may not be entirely ventricular, the auricles beating at a different rate or perhaps being quiescent. It is generally recognized that the mammalian ventricle is capable of carrying on the circulation alone, and it is quite possible this may occur in disease. In this case the rhythm may be irregular also, and the intermissions are not necessarily equal to double pulse-intervals although the ventricle alone is involved, as I showed with Matthews in our earlier article. This purely ventricular rhythm presupposes such grave disorder of the whole heart, however, that it can scarcely be regarded as of frequent occurrence, and though it would be impossible to diagnose it by the sphygmogram from the auricular irregularity, it need not be regarded as an alternative diagnosis in cases of short intermissions, until further evidence of its existence in man is adduced.

In many instances the extrasystoles or the intermissions appear regularly in the tracing, whether these be of ventricular or of auricular origin. For example, Fig. 11 (Plate XVII) is a tracing in which every second contraction for some time is an idioventricular extrasystole; in Fig. 12 (Plate XVII) every third is an extrasystole of the ventricle; in Fig. 3 (Plate XVI) every fourth is a ventricular extrasystole, while in Fig. 9 (Plate XVII) every fourth is an auricular extrasystole. This rhythmical irregularity is difficult to explain, for it is generally believed that the irritability of the heart is exhausted at every systole, so that there cannot be a summation of residual energy until sufficient is accumulated to give rise to an extrasystole. A failure of the ventricle to contract at intervals is much more readily intelligible, for it is easily conceivable that the contractile or conducting substance is exhausted entirely after every 2nd or 3rd beat, and until it is restored no contraction can occur. In many instances, however (*e. g.* Fig. 11, Plate XVII), the irregularity is obviously due to an extrasystole and not to a failure to contract. It may be added that the same phenomenon is frequently seen in animals poisoned with digitalis or its allies or with barium salts, that is, with bodies which increase the irritability of the heart. The periodic appearance of the extrasystole may therefore be due to some alteration in the heart through which the irritability is not entirely annulled by the contraction, and the residue is summed up until it gives rise to a premature systole.

A few words may be added in regard to the extreme irregularity of the heart known clinically as delirium cordis. It is unnecessary to explain that in physiology this term is used to indicate fibrillary contractions of the heart, which arrest the circulation and prove immediately fatal. The clinical sphygmogram in these cases resembles exactly that obtained from dogs when the auricle is undergoing fibrillary contractions, which may be continued for a long time without proving fatal. I do not wish to assert that the clinical delirium cordis is identical with the physiological delirium auriculæ, but the resemblance is certainly striking. The fibrillary contraction of the auricle may be induced by stimulating it by means of rapid induction shocks,

and possibly by other forms of stimulation. In some cases I have observed it in the dog before the heart was injured in any way, and in some of these cases it could be arrested and the pulse rendered perfectly regular by division of the vagi or by paralyzing their terminations by means of atropine. This connection between the inhibitory mechanism and fibrillary contractions was scarcely to be anticipated from what is known at present regarding inhibition, but is confirmed by the observation made by Matthews and by Cash that atropine prevents the irregularity and final delirium of the heart from aconitine. It would be of interest to ascertain the effect of atropine on clinical delirium cordis.

In conclusion, I have much pleasure in acknowledging my indebtedness to my colleague Dr. Dock for his kindness in putting his tracings at my disposal, and to Drs. Arneill and Boyce for the help they have afforded me in various ways, particularly in collecting tracings which bore on the subject of investigation.

DESCRIPTION OF PLATES XVI AND XVII.

Figs. 1-2 are inserted in the text and there explained (pp. 329, 330).

Plate XVI.

Figs. 3-13. Sphygmograms from human tracings. The pulse-intervals and intermissions are measured below in hundredths of a second.

Fig. 3. Every fourth pulse-beat is missed, and the intermission is practically exactly twice the normal interval, *i. e.* the ventricle alone is involved in the irregularity.

Figs. 4 and 5. Single intermissions each equal to two intervals.

Fig. 6. Single intermission shorter than two intervals ($2 \times 95 = 190$) and therefore of auricular origin.

Fig. 7. Three intermissions each shorter than two intervals. In the first (lasting $\frac{115}{100}$) and third (lasting $\frac{114}{100}$ seconds) there is evidence of a premature contraction early in the intermission, but this is absent in the second.

Fig. 8. The pulse is fairly regular at first, but, later, intermissions follow each other, each being shorter than two full intervals. In the first of these there is a distinct premature contraction, $\frac{40}{100}$ sec. after the last normal beat.

Plate XVII.

Fig. 9. Every fourth beat is missed, and each intermission is shorter than two full intervals; *i. e.* the irregularity is auricular in origin. Contrast Fig. 3.

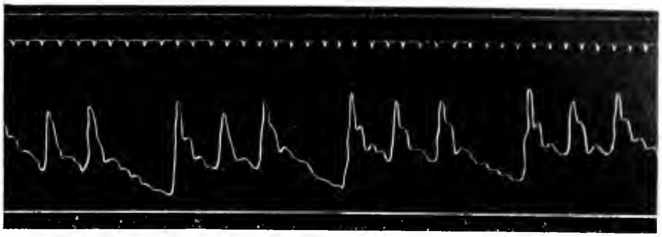
Fig. 10. Irregularity in ventricular disease (see text, pp. 338, 339).

Fig. 11. Ventricular premature systole occurring alternately with that derived from the auricle. This form of irregularity lasted for several minutes and then gave place to a regular pulse having an average interval of 64-67 hundredths of a second.

Fig. 12. Every third beat is missed, and the intermission is so nearly equal to two intervals that it may be regarded as due to ventricular disorder only.

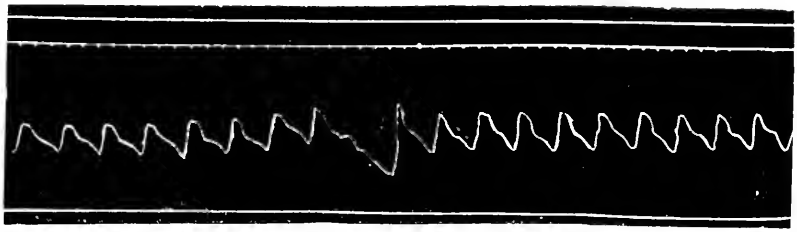
Fig. 13. An intermission caused by excessive activity of the inhibitory apparatus and not to true cardiac disease. It simulates auricular intermission.

Fig. 14. An intermission caused in the dog's pulse by stimulation of the vagus nerve for $\frac{1}{25}$ sec. Compare Fig. 13.



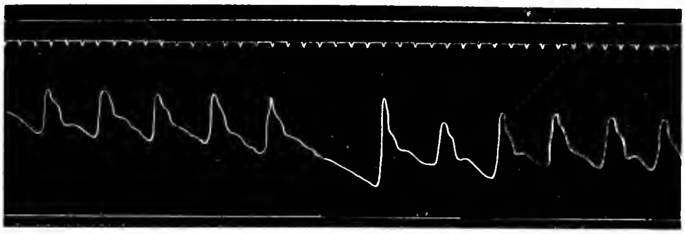
57 116 58 57 114 59 59 117 59 58 59

FIG. 3.



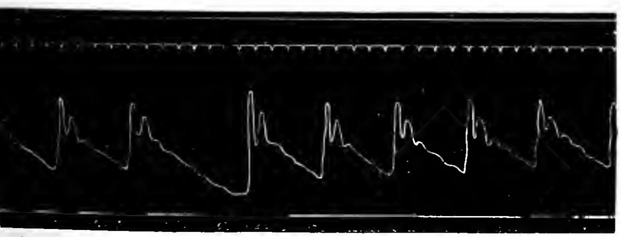
55 56 57 54 56 111 54 55 54 54 54 54

FIG. 4.



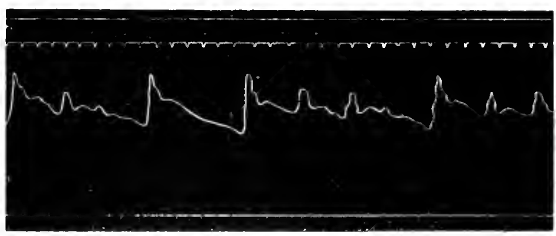
70 76 74 76 152 77 77 72 71

FIG. 5.



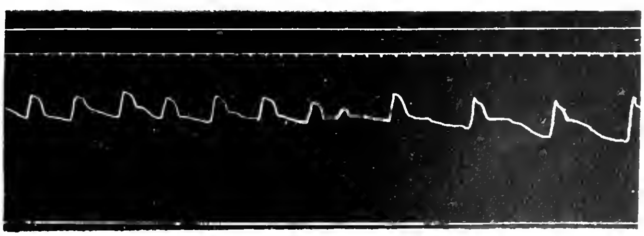
91 99 158 98 94 95 94 97

FIG. 6.



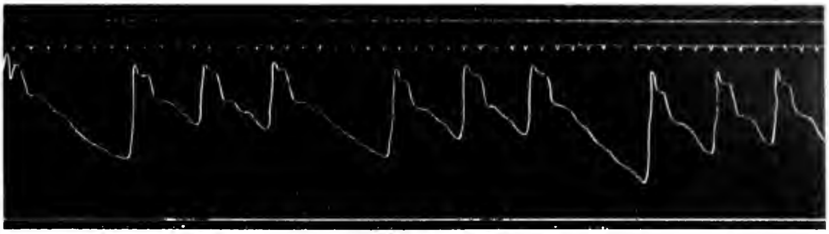
69 115 127 72 66 114 73 63

FIG. 7.



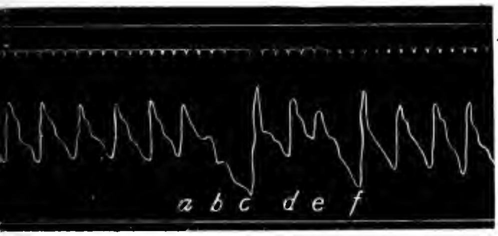
57 62 59 65 63 65 40 70 109 109 106

FIG. 8.



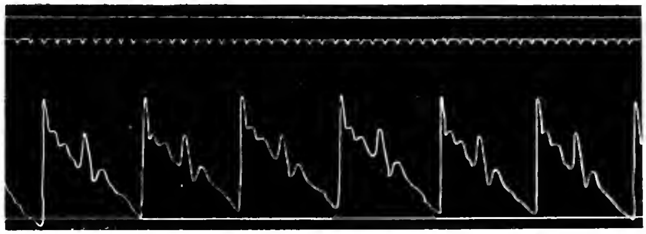
92 91 159 95 98 157 88 80 79

FIG. 9.



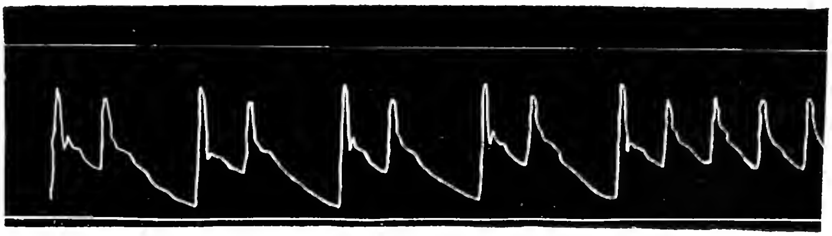
48 48 49 47 41 95 50 34 60 47 47 45 44

FIG. 10.



49 84 49 79 51 79 51 85 47 81 47 83

FIG. 11.

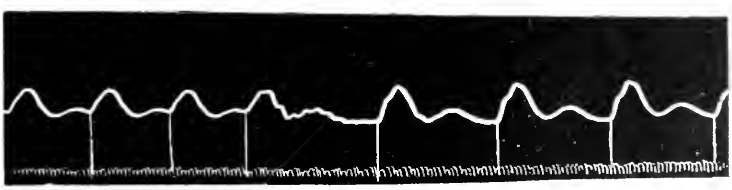


64 128 65 126 65 124 64 120 63 64 64 62

FIG. 12.

65 73 68 63 62 88 75 68 62

FIG. 13.



39 38 64 56 54 48

FIG. 14.

TABLE I.

Duration of Intermissions in the Dog's Pulse under Stimulation of the Ventricle and Auricle (see page 332).

	A. Normal Pulse-interval.	B. Chamber Stimulated.	C. Intermission.	D. (2A – C).	E. $\left(\frac{D}{A}\right)$	Average of E.
Expt. 1.	54	Ventricle.	107	1	$\frac{1}{54}$	0
	55	"	110	0	0	
	54	"	108	0	0	
	54	Auricle.	98	10	$\frac{10}{54}$	$\frac{2}{9}\left(\frac{1}{13} - \frac{2}{5}\right)$
	53	"	92	14	$\frac{14}{53}$	
	52	"	100	4	$\frac{4}{52}$	
	54	"	100	8	$\frac{8}{54}$	
	53	"	98	8	$\frac{8}{53}$	
	55	"	88	22	$\frac{22}{55}$	
	55	"	93	17	$\frac{17}{55}$	
	54	"	98	10	$\frac{10}{54}$	
	54	"	91	17	$\frac{17}{54}$	
Expt. 2.	40	Ventricle.	79	1	$\frac{1}{40}$	0
	39	"	78	0	0	
	40	"	80	0	0	
	39	Auricle.	62	16	$\frac{16}{39}$	$\frac{1}{3}\left(\frac{1}{4} - \frac{2}{5}\right)$
	38	"	64	12	$\frac{12}{38}$	
	39	"	[27+42]69	9	$\frac{9}{39}$	
	38	"	62	14	$\frac{14}{38}$	
	38	"	65	11	$\frac{11}{38}$	
	38	"	67	9	$\frac{9}{38}$	
	38	"	62	14	$\frac{14}{38}$	

TABLE II.

Intermissions which approximated the type observed in the Dog on Stimulation of the Ventricle (see page 334).

Case.	A.	C.	D.	E.	Remarks.
L.	$57\frac{1}{2}$	116	+1	$+\frac{1}{57\frac{1}{2}}$	Admitted for cataract. Pulse often perfectly regular; at other times every fourth beat was missed, as in Plate XVI, Fig 3.
	58	114	2	$\frac{2}{58\frac{1}{2}}$	
	59	117	1	$\frac{1}{59}$	
	$56\frac{1}{2}$	112	1	$\frac{1}{56\frac{1}{2}}$	
	$55\frac{1}{2}$	116	+5	$+\frac{5}{55\frac{1}{2}}$	
	55	105	5	$\frac{5}{55}$	
	56	113	1	$\frac{1}{56}$	
	$57\frac{1}{2}$	115	0	0	
	Approximate Average....		$\frac{1}{2}$	$\frac{1}{100}$	
M.	55	111	+1	$+\frac{1}{55}$	Pernicious anæmia and mitral regurgitation. Dilatation of heart, with heaving impulse and thrill with each systole, and a loud, blowing murmur (systolic) over entire cardiac area, loudest over apex. Pulse is small, quick, irregular in volume and rhythm, with occasional intermissions. (Plate XVI, Fig. 4.) Post-Mortem examination: Numerous small hæmorrhages under the pericardium. Greatly dilated right auricle, the left auricle and the ventricles being of normal size. Mitral orifice narrowed and posterior flap much shortened.
	$51\frac{1}{2}$	99	4	$\frac{4}{51\frac{1}{2}}$	
	54	107	1	$\frac{1}{54}$	
	54	112	+4	$+\frac{4}{54}$	
	55	109	1	$\frac{1}{55}$	
	$53\frac{1}{2}$	111	+4	$+\frac{4}{53\frac{1}{2}}$	
	56	110	2	$\frac{2}{56}$	
	Approximate Average....		$+\frac{1}{7}$	$\frac{1}{350}$	

TABLE II.—*Continued.*

Case.	A.	C.	D.	E.	Remarks.
H.	$62\frac{1}{2}$	126	+1	$+\frac{1}{62\frac{1}{2}}$	Admitted for recurrent appendicitis. Pulse regular except for occasional intermissions.
	$59\frac{1}{2}$	122	+3	$+\frac{3}{59\frac{1}{2}}$	
	61	120	2	$\frac{2}{61}$	
	$60\frac{1}{2}$	121	0	0	
	Approximate Average....		$\frac{1}{2}$	$\frac{1}{120}$	
Mc.	47	95	+1	$\frac{1}{47}$	Admitted for cancer of the breast. Pulse is usually fairly regular, but occasionally misses a beat. Age 56.
	46	91	1	$\frac{1}{46}$	
	$44\frac{1}{2}$	89	0	0	
	Average		0	0	
W.	$64\frac{1}{2}$	128	1	$\frac{1}{64}$	Age 22. Admitted for hernia. Heart ordinarily regular, but often misses every third beat for several minutes at a time. (Plate XVII, Fig. 12.)
	65	126	4	$\frac{4}{65}$	
	$64\frac{1}{2}$	124	5	$\frac{5}{64\frac{1}{2}}$	
	65	126	4	$\frac{4}{65}$	
	60	120	0	0	
	Approximate Average....		3	$\frac{1}{20}$	
Mg.	77	154	0	0	Age 59. Admitted for retention of urine. Occasionally the pulse misses a beat, but it is generally quite regular.
	$76\frac{1}{2}$	152	1	$\frac{1}{76}$	
	80	157	3	$\frac{3}{80}$	
	Approximate Average....		1	$\frac{1}{60}$	
B.	59	(46 + 73)119	+1	$\frac{1}{59}$	Medical student, complains of heart missing every second beat for several minutes, and then becoming regular. (Plate XVII, Fig. 11.)
	58	(45 + 70)115	1	$\frac{1}{58}$	
	60	(46 + 74)120	0	0	
	Approximate Average....		0	0	

TABLE III.

Intermissions which approximated those observed in the Dog on Stimulation of the Auricle (see page 334).

Case.	A.	C.	D.	E.	Remarks.
N.	68	(46 + 73)119	17	$\frac{1}{4}$	Age 78. Atheroma. Heart often regular for days at a time and then occasional intermissions.
	73	123	23	$\frac{23}{73}$	
	74	112	36	$\frac{36}{74}$	
	69	110	28	$\frac{28}{69}$	
	Approximate Average....		26	$\frac{3}{8}$	
Q.	93	163	23	$\frac{23}{93}$	Surgical out-patient. No history obtainable.
	97	170	24	$\frac{24}{97}$	
	Approximate Average....		24	$\frac{1}{4}$	
G.	77	123	31	$\frac{31}{77}$	Surgical out-patient. No history.
	74	129	19	$\frac{19}{74}$	
	70	(46 + 78)124	16	$\frac{16}{70}$	
	71	118	24	$\frac{24}{71}$	
	Approximate Average....		$22\frac{1}{2}$	$\frac{1}{3}$	
St. Cl.	110	179	41	$\frac{4}{11}$	Atheroma (age 66). Dulness extends to parasternal line. Sounds weak. No adventitious sounds. Radial arteries tortuous and hard. Pulse 60, irregular in volume and rhythm. Occasionally a beat is missed. (Plate XVI, Fig. 6.)
	100	167	33	$\frac{1}{3}$	
	98	158	38	$\frac{3}{8}$	
	Approximate Average....		37	$\frac{1}{3}$	

TABLE III.—*Continued.*

CASE.	A.	C.	D.	E.	REMARKS.
SH.	65	122	8	$\frac{1}{8}$	Blacksmith. Dilation and hypertrophy of the heart, double aortic lesions, mitral regurgitation and tricuspid regurgitation. General anasarca. Pulse often misses a beat. Post-Mortem examination: Great distension of all the chambers, and distinct separation of the muscle fibres in the auricle.
	72	134	10	$\frac{1}{7}$	
	69	115	23	$\frac{1}{3}$	
	71	127	15	$\frac{15}{71}$	
	66	114	18	$\frac{18}{66}$	
	Approximate Average....		15	$\frac{2}{9}$	
K.	69	118	20	$\frac{2}{7}$	Age 77. Surgical case. Pulse often misses a beat, and in the intermission a premature pulsation is sometimes present (Plate XVI, Fig. 8). Sometimes the intermission occurs alone in a series of regular beats. In other tracings a number of consecutive intermissions occur.
	58	100	16	$\frac{16}{58}$	
	57	100	14	$\frac{14}{57}$	
	65	110	20	$\frac{20}{65}$	
	63	104	22	$\frac{1}{3}$	
	62	105	19	$\frac{19}{62}$	
	64	110(40 + 70)	18	$\frac{1}{4}$	
	69	114	24	$\frac{1}{3}$	
	Approximate Average....		19	$\frac{5}{16}$	
D.	81	147	15	$\frac{15}{81}$	Acute rheumatism. Dilatation of the heart towards left. First sound is short and accompanied by a soft blowing murmur. Over the pulmonary area is heard a soft blowing murmur with the first sound. Radial pulse moderately strong, 75, quick, at times every third beat is dropped (Plate XVII, Fig 9), and then again the heart becomes more regular or the intermissions appear at longer intervals. Heart improved under salicylic acid treatment.
	$81\frac{1}{2}$	145	18	$\frac{18}{81}$	
	83	148	16	$\frac{16}{83}$	
	83	147	19	$\frac{19}{83}$	
	81	149	13	$\frac{13}{83}$	
	Approximate Average...		16	$\frac{1}{5}$	

ON THE DIPLOCOCCOID FORM OF THE COLON BACILLUS.*

By J. GEORGE ADAMI, M. A., M. D., F. R. S. E.; MAUDE E. ABBOTT, B. A., M. D.; AND F. J. NICHOLSON, B. A., M. D.

(*From the Pathological Laboratory of the Royal Victoria Hospital, Montreal.*)

PLATES XVIII-XX.

In the course of a careful study of a long series of livers, both cirrhotic and otherwise, we have in the specimens examined, with scarce an exception, encountered larger or smaller numbers of minute bodies, and the more we have studied them the more assured we have become that these are bacterial in nature. Under the ordinary 1/12th immersion lens and by the usual methods of staining these may easily be overlooked and, if recognized, they may easily be mistaken for minute pigment granules present in the liver cells. But by more intensive staining and by employing a good 1/18th immersion lens, their nature becomes more evident.

The methods we have employed with the greatest amount of success have been by staining with carbol-fuchsin (one-half the ordinary strength) and subsequent bleaching in the sunlight in our earlier observations; and, of late, almost exclusively carbol-thionin, made according to the formula recommended by Muir and Ritchie,† the sections being cleared by aniline oil. Stained by either of these methods the granules resolve themselves in the main into fine diplococci surrounded often by a fine halo as to the nature of which we shall speak later. When these diplococci are present in any numbers there may also be isolated minute spherical and ovoid bodies of the same dimensions and there may also be seen occasional strings of three or four coccus-like bodies.

* Read at the meeting of the Association of American Physicians, Washington, May, 1899.

† Muir and Ritchie, Manual of Bacteriology, Edinburgh and London, 1899, p. 109.

We have recognized these in the livers of man, the cow, sheep, rabbit, and guinea-pig. At first, working with the cirrhotic livers of cattle and of man, one of us was inclined to regard these as peculiar to cirrhosis, but, as already announced (1), fuller study having shown their existence in the apparently normal liver, they cannot be regarded as specific of any one disease, although it is possible that they are one factor in the production of certain forms of fibrosis. Under these conditions they tend to take on a relatively deep stain, but in the majority of cases they stain badly, have a characteristic brownish tinge and would seem to be dead.

From several cases of cirrhosis in which these were recognizable, cultures gave either vigorous, or what we must now regard as attenuated, growths of a colon bacillus, while after intravenous inoculation of adult rabbits with 48-hour broth growths of our stock culture of the colon bacillus, which is in every respect typical, the liver cells showed these minute diplococcus forms in enormous numbers.

We were therefore led to conclude that, while it might be that other bacillary forms may also show a diplococcus-like appearance in the tissues, we had adequate evidence that the colon bacillus can show this appearance, and during the last few months we have conducted a long series of observations bearing more especially upon this diplococcus-like modification of the bacillus. Our work is divisible into two portions:

I. On the production of a diplococcoid form of the colon bacillus outside the organism.

II. On the diplococcoid form of the bacillus within the tissues.

The former portion has been undertaken in part by Dr. Nicholson, the latter portion and the studies upon growths in body fluids by Dr. Maude E. Abbott.*

* But for the fact that, by my previous publication on the subject, I have made myself peculiarly responsible for these observations upon the colon bacillus, I would very gladly have left my own name off the title page; for, in consequence of prolonged absence from my laboratories, the observations have been throughout conducted by Dr. Abbott and Mr., now Dr., Nicholson, and I cannot sufficiently acknowledge the enthusiasm which they have thrown into the work. J. G. A.

PART I.

ON THE PRODUCTION OUTSIDE THE BODY OF A DIPLOCOCCOID FORM OF THE COLON BACILLUS.

While under ordinary conditions of growth outside the body and ordinary staining, by Löffler's blue for example, the colon bacillus is an undoubted bacillus with no recognizable internal organization, it has been a matter of frequent observation that it might present distinct polar staining, and indeed, when stained by fuchsin or other strong reagent for purposes of photography this so-called polar staining is very conspicuous. We need but refer to the various published photographs to confirm this statement. In these photographic reproductions of film preparations from cultures, the majority of the bacilli are seen to be present as two rounded coccus-like bodies lying in close apposition, a common enclosing or joining sheath being more or less clearly evident.

The appearance here observed is that which is generally spoken of as "polar staining." It is common to a large number of bacteria and, in not a few cases, as for example among the bacteria of hæmorrhagic septicæmia, has led in the past to not a little confusion in descriptions, authorities having been divided as to whether to class bacteria exhibiting the property in a marked degree as bacilli or diplococci. In certain cases, as in connection with the typhoid bacillus, it has been attributed to a retraction of the protoplasm to the poles during the process of preparation and staining of the film of bacteria—and thus has been regarded as an artifact.

We shall not here enter into the discussion concerning polar and metachromatic granules, but simply state that our observations, so far as they go, would seem to negative this latter supposition and to render it evident that in the case of the colon bacillus at least, *there is a structural condition or internal organization of the microbe underlying and explaining such polar staining.* What is more, they show us that the appearances seen in the colon bacillus are closely allied to the "beading" to be made out in the tubercle bacillus under certain conditions of growth and environment.

As already pointed out by A. Schmidt (2), Rodet (3) and others, the colon bacillus varies according to the length of time it is kept outside the body, according to the mode in which it is grown, the reaction of the medium, the temperature, and the part from which it has been isolated. Rodet has found that when it is taken from the healthy intestines the individuals during the earlier generations outside the body are singularly even in length and thickness and stain well throughout; when taken from diseased tissues—from the inflamed gall bladder, for example—this is no longer the case; they are irregular both in length and thickness, they stain irregularly and show clear spaces and deeper staining portions.

Rodet points out that a temperature of 44-45° C. leads during the first few hours to the appearance of very long filaments, though other individual forms are of the normal length. All these filaments show refractive bodies which take up intensely the basic aniline color. After 24 hours these long filaments disappear. In addition, according to this author, growth upon broth containing 2.5 per cent lactose leads to the production of peculiar short and small forms almost like cocci, the majority of which are double and in the form of diplococci.

These observations of Rodet have just come into our hands and we can in the main confirm them; indeed, in ignorance of this work published two years ago, we have been working very much along the same lines as those indicated by Rodet; who, however, it may be added, has noted these appearances without studying more fully their nature.

We find that the long filaments mentioned by Rodet are to be observed in cultures kept for a few hours at a high temperature. It must not, however, be thought that they are exclusively confined to this period. Similar long filaments showing even more clearly the presence of deeper staining bodies within them, are to be gained from old cultures associated with involution forms. Thus in a specimen of our stock colon bacillus grown for a fortnight in broth containing a trace of bile, we found great numbers of these long bodies and, associated with them, large numbers of a small diplococcus form. Perhaps the most interesting of these long filaments were observed in an agar plate culture obtained from the spleen in a case of cirrhosis which

had been subjected for a few hours to a temperature of about 45 or 46°; removed from the incubator, this had grown under difficulties (brought about by the partial drying-up of the medium) for four days at the ordinary temperature. In this the disposition of the deeper staining points was remarkable (vide Fig. 8, Plate XVIII). Seen under the 1/18th immersion lens, after staining by carbolic fuchsin and decolorizing by weak acetic acid, these fine deeper staining points were arranged in a succession of pairs with occasional larger single ovoid bodies interposed. We have come across one other specimen of a rather prolonged growth in which the same appearance was recognizable though not quite so clearly. Possibly the exact extent of the staining and subsequent decolorization may have something to do with the difficulty in recognizing this particular arrangement of the contained bodies.

We have also found that taking saliva, filtering and sterilizing it, and making cultures in this medium at the ordinary temperature, we obtain the production of these long filaments, which may be present in the growth not only during the first 24 hours but during the continuance of the culture.

Under these conditions in the saliva of one of us (F. J. N.) the bacilli were throughout singularly slim and in the later growths again they tended to show the development within the bodies of the bacilli of a succession of deeply-staining dots.

A. Schmidt has noted that he obtained these filamentous forms of the colon bacillus by the addition of caustic soda to broth. We found that we obtained the longest forms by employing lactose broth rendered 1.5° acid * to phenolphthalein and containing 2.5 per cent lactose. Here more especially on the surface exposed to the air at the end of 24 hours we obtained remarkably long filaments. Indeed, we cannot agree with Rodet that the addition of this relatively large percentage of lactose to broth results in the production of the diplococcus forms. It is a misfortune that Rodet did not state more precisely the composition and the reaction of his broth.

* According to the standard given in the report of a committee of American Bacteriologists, 1898.

In order to obtain the diplococcoid forms of the bacillus we conducted a series of experiments upon growth in broth of varying degrees of alkalinity and acidity at a temperature of 46°. Under these conditions it seemed certain that after the first 24 hours we obtained, more especially in slightly acid broths, a relatively increased proportion of short forms with polar staining, but we could not convert all the bacilli into the diplococcoid form. It was when we attempted to grow the bacillus upon certain of the body fluids that we met with the greatest amount of success.

The frequency with which we had encountered this diplococcus form in our observations in the liver made us wonder whether our method of gaining cultures might not have been, in part at least, accountable for the phenomenon. As Livingood (4) has shown, growth of the colon bacillus upon organic juices expressed from the liver, spleen, etc., has some slight effect upon the morphology of this microbe. He noted that while the colon bacillus in general was relatively very large when grown upon *heated* liver juice, upon *unheated* he obtained very short, thick, almost oval forms with abrupt ends, these occurring occasionally in pairs. Here in the development of these oval forms there is, it may be urged, an approach towards our diplococcoid form, but so careful an observer would have made a fuller note upon the subject had he recognized constantly the development of the diplococcoid appearance.

But it must be pointed out that there is a difference between inoculating a medium with a loopful of a culture, *i. e.* with hundreds of thousands of a micro-organism, and employing a medium in which what bacilli are present have gained an entrance through the ducts and excretory channels of the organ from which the fluid has been obtained. Working with bile for example, we have frequently found that by making ordinary streak cultures in the usual method we obtained no results, whereas, gaining the bile direct from the bladder by means of a pipette and adding a drop or two of this to broth, growths were obtainable.

The conclusion which we have reached is that in such cases the bacteria have been present in relatively small numbers, numbers so

small that the somewhat weak inhibitory action of the bile has been sufficient to prevent growth when this bile has not been diluted. All our work goes to show in fact that bile has a slight inhibitory effect, not necessarily destroying the micro-organisms, but permitting growth to continue under unfavorable conditions and it is under these unfavorable conditions that we have obtained either absence of growth or development of the diplococcoid form. For example, we have noticed in several cases that whereas with bile taken immediately from the body we have obtained no cultures, when a pipette of that bile has been kept for several days in the incubator, fairly numerous fine colonies of the B. coli have developed in which the individuals show a tendency to assume the diplococcoid form. These observations prepared us to find that the diplococcoid form of the bacillus might be a modification brought about by the action of the body fluids; but more especially were we led to employ these body fluids by two interesting observations.

In September, 1898, our attention was called by Dr. W. F. Hamilton to a case of what was diagnosed as atrophic cirrhosis in the medical wards of the Royal Victoria Hospital; this diagnosis was subsequently fully confirmed at autopsy. Through the kindly interest of Dr. Hamilton we were present at the first tapping of the patient and then obtained under careful antiseptic precautions sterilised flasks of the ascitic fluid; at the same time a guinea-pig was inoculated with 10 cc. of the same fluid and cultures were made directly upon broth, agar and blood-serum.

A full account of this case is on the point of publication by one of us and we will here give only a brief epitome of the results.

Upon agar and Löffler's blood-serum, there developed scattered small colonies of a form which at first was taken to be a diplococcus but which later, in the course of 48 hours, upon these media as in the broth, showed the presence of definite stumpy bacilli, often arranged as short diplo-bacilli, in fact, the form which we recognize as very characteristic of the colon bacillus. Unfortunately vacation time came on and the opportunity to examine fully these forms passed by. However, the guinea-pig died in 24 days, the autopsy was performed

a few minutes after death and from all the organs we obtained a pure culture of the colon bacillus which appeared to be quite typical. Among the organs from which cultures were made was the gall-bladder; this gave a pure culture of the colon bacillus.

A pipette full of the bile of this guinea-pig, which possessed the characters dwelt upon by Welch and Blachstein (5), *i. e.* was clear, abundant and of a light color, showed even when placed in the incubator no apparent growth but remained unclouded; at most a few fine granular flocculi were present after some days. But upon examining a film of this bile which had thus been kept, it was found to contain abundant minute diplococci (vide Fig. 12, Plate XVIII). These grew easily when transferred to agar, the colonies being minute and much smaller than those of the typical colon bacillus.

From the first transfer upon broth, coccus and diplococcoid forms predominated with occasional homogeneous stumpy bacilli (vide Fig. 1, Plate XVIII). Later transfers upon agar from this broth led to the development of the typical bacillary form—stumpy bacilli with rounded ends, often arranged as short diplo-bacilli and showing a tendency towards polar staining. The morphological features of the cultures now became coarser and resembled those of the ordinary colon bacillus.

Evidently, therefore, the bile of the guinea-pig exercised an inhibitory effect upon the growth of the colon bacillus and this in two directions: In the first place the growth was peculiarly slow, so that the bile did not become turbid; in the second, the individual bacilli were distinctly modified, they were very much smaller than normal and stained in such a way that they might easily be mistaken for minute diplococci. In fact the resemblance between these minute diplococci and the minute diplococcus forms seen both in the cirrhotic and the normal liver is most striking.

What is true of the bile would seem equally true of the ascitic fluid taken from this case of cirrhosis. The fluid obtained was slightly opalescent; and upon keeping, there gradually separated out a thin, gelatinous, proteid precipitate. Placed in the incubator, the fluid remained clear, and for the first few days appeared to be sterile; by the

end of a fortnight, however, a granular deposit was distinguished and now examination of the fluid showed the presence in it of singularly minute diplococci tending to be arranged in chains (vide Fig. 2, Plate XVIII). It may be remarked that this chain-like arrangement of the colon bacillus has been previously observed by Dunbar (6), Schmidt and other workers.

Between September 3 and October 13 no less than five tappings were made, of which the third and fifth were subjected to examination. Both of these gave cultures upon broth and agar showing diplococci merging into stumpy ovoid forms. Here again, cultures were made immediately from the ascitic fluid which showed forms of the colon bacillus, but the ascitic fluid kept in the incubator presented only pure cultures of an extremely minute diplococcus. After keeping for 3 weeks, subcultures upon agar made from the ascitic fluid no longer gave the typical colon form; instead of this, a modified form was obtained, the individuals remained relatively small and very short (vide Fig. 5, Plate XVIII); only after prolonged subculture and successive inoculations from 1 per cent glucose broth did the forms become slightly larger and developed into a stumpy diplo-bacillus smaller than the typical colon bacillus. What is more, they did not induce fermentation of glucose or dextrose broth or cause the indol reaction. It must be pointed out that by this process of successive cultivation through glucose broth, the form which was a characteristic diplococcus had become converted into a small bacillus arranged as a diplo-bacillus and this stained homogeneously.

Upon passage through three guinea-pigs (the guinea-pigs being killed from twelve to twenty-four hours after intraperitoneal inoculation) and growth upon 2.5 per cent lactose broth, the form has become still larger and more typical but still we fail to obtain gas production (vide Fig. 6, Plate XVIII).*

Within the last few days we have again obtained this diplococcus form from the human body. The patient, under Dr. Garrow in the

* Since reading this paper at Washington we have obtained similar results with the ascitic fluid from another case of atrophic cirrhosis in the service of Dr. W. F. Hamilton, in which again the diagnosis was confirmed at the autopsy.

surgical wards of the Royal Victoria Hospital, suffering from marked biliary crises, was operated upon in the expectation of finding a condition of cholecystitis with gall-stones. Upon opening the abdomen, a small amount of fluid escaped and a platinum loop of this was smeared upon agar-agar, which remained sterile, and immediately about a drachm of the fluid was collected under strict aseptic precautions in a sterile flask and brought to the Pathological Laboratory. Here this was added to about an equal quantity of sterilised broth and placed in the incubator. Upon continuing the operation, the gall-bladder and ducts were found pervious; there was however a condition of perihepatitis with subacute peritonitis affecting the upper half at least of the abdominal cavity and with this was associated some thickening of the great omentum.

Upon examining the above-mentioned broth culture after 24 hours, Dr. Brown, the resident surgeon, found that it contained a pure culture of minute diplococci and immediately called our attention to it. In the features of this growth upon various media, this form has so far been found to resemble the minute diplococcus already mentioned as obtained from the case of cirrhosis, though the growth is slightly more active and free. Passage through guinea-pigs and lactose broth has resulted in the development of a form identical with that just mentioned (vide Fig. 7, Plate XVIII).

There is very slow development of turbidity in ordinary broth, rather more rapid in glucose broth, but with an absence of any sign of fermentation. The growths upon the surface of agar in both were at first singularly fine so that they resembled closely those of the Streptococcus pyogenes, though possibly more transparent than the latter. Upon potato the growth was invisible; upon blood-serum the colonies were also very fine and were of an opaque white fading to a yellow tinge. Upon gelatine there was slow growth without liquefaction, while litmus milk was decolorized until it became almost perfectly white, then slowly in the course of the 5th day or so a fine pink color was developed in the medium; the milk was coagulated at the end of a week. Growth upon broth was definite but not abundant and was associated with singularly little turbidity, a white somewhat stringy

precipitate being slowly formed. In the fermentation tube the open limb became opalescent or moderately turbid in the course of 48 hours, the closed limb remained perfectly clear, and, in addition, in neither glucose nor in lactose is there any production of gas; further, there was and is no indol reaction, and if turbidity be present it is still singularly slight.

It is unnecessary here to describe all the methods that we have employed in order to cause these forms to revert to type. Briefly, we may say that we have obtained the greatest change by culture for 24 hours upon broth rendered 1.5° acid, according to the method recommended by the Committee of Bacteriologists, to which 2.5 per cent of lactose has been added. In this medium already at the end of 24 hours there is abundant growth and well-developed turbidity, and the individual forms are relatively large and ovoid, frequently arranged as stumpy bacilli (v. Figs. 6 and 7, Plate XVIII).

When this form is inoculated into the guinea-pig intraperitoneally and cultures made from the peritoneal fluid at the end of 9 hours, both upon agar and glucose broth, growth upon glucose broth in the fermentation tube is much more active than before inoculation; and whereas, previous to inoculation, only the open end of the tube had been rendered opalescent, now there is turbidity throughout both tubes. As already stated after passage through three guinea-pigs and growth of this same medium the form produced is undistinguishable from the normal colon bacillus.

It is possible that this remarkable and somewhat persistent diplococcoid form, obtained both from the bile of the inoculated guinea-pig and from the ascitic and peritoneal fluids, has become attenuated during its stay in the body; and, in the case of the bile for example, during the passage through the liver the colon bacilli have been markedly modified. We have taken sterilised human bile and added to this a minute quantity of a stock culture of the colon bacillus and have not been able to obtain in the bile the diplococcus form alone, although it is true that diplococcus forms have been relatively abundant.

Here it is interesting to recall a point which we again find observed

by Rodet, namely, that the human bile has a distinct inhibitory effect upon the multiplication of the colon bacillus. Bile, to which a minute drop of a twenty-four-hour-old culture had been added, remained to all appearances perfectly clear and apparently no growth had occurred during four days; but when a drop of this bile was added to about 10 ccm. of slightly alkaline broth and placed in the incubator, that broth rapidly became turbid, and there was most abundant development of the bacilli. We are making further observations upon this modification of the bacillus by growth in bile. This, however, may be said at the present time: that possibly the existence of bacteria in the bile may easily be overlooked when the ordinary methods of culture upon solid media are employed, the concentrated bile inhibiting their growth.

One of us (M. E. A.) has already found that human bile (3 cases), which was apparently sterile when streaked upon agar-agar, gave abundant cultures of the colon bacillus when a small drop was added to about 10 ccm. of glucose broth.

CONCLUSIONS.—PART I.

1. The short form of the normal colon bacillus, cultivated upon the ordinary bacteriological media, frequently presents polar staining; the appearance given being that of two rounded bodies staining more deeply than the rest of the bacillus and lying in and united by less deeply staining material.

2. In the more filamentous forms a succession of these more deeply staining bodies is at times to be recognized.

3. Growth outside the body under relatively unfavorable conditions renders the polar staining more prominent, so that the shorter forms may closely resemble diplococci, and the filamentous forms show a common unstained or lightly staining sheath in which is to be made out a succession of minute dots in pairs, and of somewhat larger more ovoid dots.

4. We have so far been unable by modifying the reaction of ordinary media and by continued growth at a high temperature (46° C.) to produce cultures in which the diplococcoid form alone has been present, although by these means we have gained cultures in which this form has predominated.

5. On the other hand certain body fluids sown naturally, if we may so term it, with the colon bacillus—*i. e.* the ascitic and peritoneal fluids, from a case of hepatic cirrhosis and of peritonitis respectively, and the bile of a guinea-pig inoculated with an attenuated (?) form of the colon bacillus—have yielded us diplococcoid growths so modified that we have not so far been able to cause them to revert completely to "type."

6. It has been by the prolonged action of these fluids that these changes in the colon bacilli have been produced; cultures made from them immediately after removal from the body have yielded us, either immediately or after one or two transfers, typical cultures of the colon bacillus; where these fluids have been kept from ten to twenty days the modified diplococcoid form has been produced.

7. The slight but definite inhibitory action of bile upon the growth of the colon bacillus is shown in two ways: (*a*) Streak cultures of bile upon agar may remain sterile, whereas the same bile added to ordinary peptone broth may be the seat of active growth. (*b*) Similar bile kept for several days in the incubator remains clear and shows singularly little evidence of growth within it, while subcultures from this yield fairly numerous colonies of a modified diplococcoid form of the bacillus.

8. The ascitic fluid from a case of hepatic cirrhosis was found to possess similar properties of modifying the colon bacillus and inhibiting its growth.

9. These modified colon bacilli are relatively minute, assume a diplococcoid form, are non-motile, form pin-point colonies upon agar-agar, cause but slight turbidity in broth and an almost invisible growth upon potato, act but slowly upon litmus milk, have lost the power of fermenting glucose, lactose and dextrose broths, and do not develop the indol reaction.

PART II.

ON THE DIPLOCOCCUS-LIKE MODIFICATION OF THE COLON BACILLUS IN THE TISSUES.

Taking a series of four young rabbits, weighing from 225 to 305 grms., we inoculated into the marginal vein of each 0.75 ccm. of a

24-hour growth of the colon bacillus and killed the animals at intervals of 15 minutes, 30 minutes, 1 and 2 hours. The various organs were immediately placed in formol-Müller and were subsequently cut in celloidin and paraffin, the sections being stained by carbol-thionin.

Our attention was at first especially directed to the liver. Here already in the animal killed 15 minutes after intravenous inoculation, a definite series of changes was seen to have occurred (vide Figs. 13 and 14, Plate XIX). In the blood vessels of the liver free bacilli of normal size and appearance were occasionally to be observed but, already, bacilli could be recognized within the leucocytes in the blood stream. The number of these leucocytes was not excessive but each contained a relatively large number of bacilli. In addition, already the endothelium lining the vessels was seen to be very prominent; here and there these cells contained a fairly large number of bacilli.

In 30 minutes the number of bacilli in the endothelial cells and the number of endothelial cells containing bacilli was markedly increased. These bacilli situated within the endothelial cells already showed strongly marked differences from those free in the blood stream. The latter were of normal length and thickness and took on a homogeneous stain. Those within the endothelial cells were short and stumpy, sometimes almost coccus-like. The appearance given is that of a primitive bacillus having been broken up into shorter lengths.

In the rabbit killed at the end of one hour, the number of bacilli seen in the blood stream was distinctly less; but there was a further increase of those in the endothelial cells. Occasionally in the endothelial cells relatively large bacilli could be seen, but the majority of forms were, as in previous specimens, very short and stumpy, and the impression gained by a study of the sections is that the bacillus is taken up in the long form and subsequently broken up into shorter sections. At this period no well stained bacilli could be seen in the liver cells. Already in the endothelial cells certain of these stumpy forms had the appearance of diplococci of fair size.

In the liver of the rabbit killed two hours after inoculation the same appearances were to be made out as those seen in the rabbit of one hour, namely, the presence of short and stumpy bacilli in the en-

dothelial cells; we were of the opinion that a larger proportion of these had the appearance of diplococci than in the previous sections. In several places between the liver cells, as indeed also in sections taken at an earlier period, there were to be made out apparently within the vessels hyaline masses which contained numerous bacilli. We have found some little difficulty in coming to a conclusion as to the nature of these masses; the large ones would seem certainly to be hyaline thrombi, but in the smaller ones it was often difficult to make quite certain whether we were not dealing with some phenomenon in connection with the endothelial cells; for very frequently a nucleus of endothelial type was in close connection with these smaller hyaline masses. We could not absolutely leave out of account the possibility that we were dealing with very greatly swollen endothelial cells.

Up to this point we were unable to recognize in any of the sections of this series indications that the bacilli had been taken up by the liver cells. But in a rabbit killed four hours after inoculation we came across great numbers of extremely minute brownish shadows definitely within the hepatic parenchyma (v. Fig. 15, Plate XX). We have been wholly unable to stain these little bodies and indeed only by very careful examination with the 1/18th immersion lens have we been able to see them distinctly, but with this magnification there they most certainly are and the more carefully they are studied the more clearly they are seen to be present in general as extraordinarily minute little brownish diplococci, at times showing a halo around them. And the more one has studied these appearances the more it seems likely that this apparent halo indicates that these small bodies lie in vacuoles, although in part also the appearance may be due to the existence of an unstained sheath or body substance.

Evidently, judging by the sections from this stage of the inoculation disease, not only are the bacilli taken up in large numbers into the liver cells, but being taken up they undergo rapid digestion and destruction so that they can no longer be stained by the ordinary methods and what we see are essentially the shadows of the bacilli. We have attempted to make out the stages by which the bacilli pass from the endothelium into the liver cells, but so far without great success.

Here and there in sections of the two-hour rabbit we have been able to make out that the endothelium appeared to be raised from the underlying cells and on the inner side of this endothelium very rarely we could see in the spaces between the endothelium and liver cell, well-stained coccus or diplococcus-like bodies. We are, however, unwilling to dwell too strongly upon these appearances, inasmuch as the endothelial cells showing these features were crowded with bacteria and we could not exclude the possibility that in the process of preparation the cells might have become slightly dislodged and that so the appearance of the bacilli apparently outside the main body of the cells might be due to their presence in a slightly different plane.

Taking next well-developed rabbits similarly inoculated and killed at the end of 24 hours, we have found in them the presence of bacilli in the endothelial cells, while the brown shadows, as we may term them, have been present in enormous numbers in the liver cells.

Thus far then, from what we have said, it would appear evident that when the colon bacillus enters into the circulation it is liable to be taken up rapidly by the endothelial lining of the hepatic vessels and in this process undergoes division into smaller segments; so that in the main one meets with stumpy forms in these cells, forms which still stain well, although often showing a tendency towards a diplococcoid appearance. Following upon this within 4 hours these bacilli are discharged by the endothelial cells and are by some means or other taken up by the hepatic cells, and there are rapidly destroyed, so that it is only by careful examination that minute coccus or diplococcus-like bodies are discovered within the liver cells.

It is interesting to note that upon examining a film of the bile taken from inoculated animals at the end of 24 hours one can, by careful preparation, recognize in it these very minute diplococcus-like bodies. To see them it is necessary to make a very fine film, treat with weak acetic acid, wash, and then stain with dilute carbolic fuchsin and examine under the highest power.

But we now come to certain great difficulties in connection with the statements here made. In the first place, making a large series of control observations upon the livers of apparently normal adult rab-

bits we have frequently come across these same diplococcus-like bodies and in four instances in relatively very great numbers. Indeed, these diplococcus-like bodies would seem to be very frequently present, more often present than absent from the rabbit's liver.

To obviate this difficulty it seemed to us that we might obtain more decisive results by employing very young rabbits, from 3 to 6 weeks old. In our control of the livers of these very young rabbits, we have found that the diplococci appear to be absent. Upon making a like series of inoculations into these very young rabbits and killing at 2, 4 and 24 hours, we hoped definitely to settle the question. But here at first we had wholly negative results. By our routine methods of staining we were unable to detect any bacteria within the cells even when we employed sections that had been cut in paraffin. So opposed to all our previous results and conclusions did this appear that for a time we were on the point of relinquishing this communication. It is possible that either the carbol-thionin used by us for the experiments was defective, or our technique modified in some slight degree, for at the best the carbol-thionin method does at times show itself wanting. But our failure was so constant that we hardly believed that this explanation would suffice.

Now we have attempted to stain other sections from the same blocks by other methods and we eventually found that staining for half an hour with Löffler's methylene-blue, washing with tepid water, and then passing through absolute alcohol and xylol, we obtained sections in which the tissue is relatively faintly stained and in which we were able to detect within the cells peculiar small diplococci having the faintest brown tinge. These were obtained from the livers of animals which had been inoculated two and four hours before death. Our failure to recognize these bodies is in fact due to their minute size and their very faint stain. We have examined control livers also from young animals by the same methods with negative results.

It would seem clear to us that the rate at which the colon bacilli are taken up and destroyed in the liver varies to some extent in different animals, according to the condition of the tissues and the virulence of the microbe. It is to be noted that the culture employed in this

latter series was from the same stock as that employed previously—a stock which had been grown outside the body for now an additional six months. And here we may notice that the diplococci staining most deeply and also those having the deepest brown tinge were in the livers of rabbits dying from 3 to 4 weeks after inoculation as again in certain of our control animals. Our experimental animals which had been kept alive at the most for 24 hours have yielded us only diplococcus forms showing but a delicate brown staining within the cells.

While we were in doubt with regard to this second series of livers, it seemed to us well to study another excretory organ not in connection with the portal circulation. Examining the kidneys of several control rabbits, we have in no case been able to find the diplococcus forms present within the organ in the great numbers in which we have come across them in the livers of the same animals. We have met with occasional diplococci within the cells of the convoluted tubules, but these have been rare. We have found that the examination of the kidneys for these modified colon bacilli has been a matter of considerable difficulty. Undoubtedly they are taken up by the cells of the convoluted tubules. Of this we have abundant evidence, and occasionally we have come across well-staining diplococcus-like forms in the outer portion of the kidney cells, but the diplococcus forms appear to be destroyed with great rapidity and in the process of destruction do not assume the brownish tinge already referred to in connection with the liver; thus it has been a matter of extreme difficulty to trace them. We have, however, seen them in great numbers in the cells of the convoluted tubules of the rabbit 2 hours after inoculation and again at 24 hours, in this latter the number being greater. Here also in the cells of the tubules in very thin sections we have come across numerous minute vacuoles of an elongated oval shape often slightly dented in the middle, and within these we have at times been able to distinguish two very minute dots, evidently the very final indication of the disappearing and destroyed bacillus (vide Fig. 11, Plate XVIII). Independently, Dr. A. G. Nicholls (7) has studied the kidneys of the animals inoculated by us and has met with these diplococcus forms, fully confirming what we have stated.

CONCLUSIONS.—PART II.

Our observations, therefore, upon the rabbit would lead us to the following conclusions:

1. That the colon bacillus injected into the circulation is rapidly taken up both by the liver and the kidney.

2. That within 15 minutes after inoculation some bacilli are already ingested by the endothelial cells in the liver, this process of ingestion continuing until some of these cells are full of bacilli.

3. That in this process of ingestion the bacilli are broken up into shorter lengths and that these short stumpy bacillary forms may already within the endothelial cells present themselves as two deeply-staining dots and may thus resemble diplococci.

4. That already in two hours the modified bacilli may be discharged outwardly from the endothelial cells and be taken up by the underlying liver cells.

5. The exact stages of this discharge we have been unable to follow. In the liver cells the modified bacilli are to be recognized as small diplococci of a size varying from that equal to the diplococci seen in the endothelial cells, down to points of extreme tenuity:—evidently these forms are undergoing destruction. In the first place, they lose their power of staining; in the second, if the destruction is not too rapid, they assume a brownish tinge. The causation of this brownish tinge we have not yet determined, but it is to be made out in the unstained sections, and our studies upon the human liver indicate to us that not a little of the fine pigmentation common in liver cells is brought about by the existence in these cells of these minute elements of bacterial destruction.

During this process of destruction the modified bacilli lie in digestive vacuoles and the frequent appearance of the halo around these forms is in great part due to the existence of the vacuole. We have occasionally been able to make out what appear to be these vacuoles in the liver cells without the evidence of the contained microbe, that having been apparently entirely digested. We have seen the same appearance also in peritoneal leucocytes nine hours after intraperitoneal inoculation with modified colon bacilli (vide Fig. 10, Plate XVIII).

6. In the kidney the same process is at work—we have recognized the diplococcus form within the cells at the expiration of two hours after inoculation and have also seen the vacuoles within the cells and convoluted tubules and there have occasionally met with the two dots just visible as final indications of the process of digestion of the bacillus.

We sincerely hope that others will repeat and confirm these observations, though to those repeating them, we would point out that it is absolutely essential to employ higher powers than those ordinarily used for bacteriological investigations, while the finest sections are requisite to give clear results. Very careful technique in the matter of intensive staining and decolorizing of the tissues is also an essential. Unless these points are attended to, our frequent difficulties in forging the chain of evidence here brought forward, will certainly present themselves and, without great patience, we cannot expect others forthwith to corroborate our results. We are prepared, that is, to find these results called in question. But after many months' and, on the part of one of us, many years' puzzling over these peculiar pigmented bodies seen more especially in the liver, we do not see what other conclusion to reach. Here we may say that we are prepared to find bacteria other than the colon bacilli, when taken up by the cells of the liver and kidney, assume very similar forms. Indeed, we already have evidence of this in connection with the typhoid bacillus.

If the above conclusions are correct, it is clear, judging from what we have said concerning the appearances seen in many normal livers of rabbits—and seen also, we may add, in the human liver—that the liver as an organ possesses the most important function of taking up and destroying bacteria, more especially the colon bacillus, which have gained admission through the portal blood, while the kidney possesses a like power of destroying rapidly bacteria circulating in the general systemic blood.

As our paper is more especially upon this diplococcoid form of the colon bacillus and its modifications within the body, we will not here dwell upon this subject, especially as one of us has already called attention to this conclusion elsewhere (1).

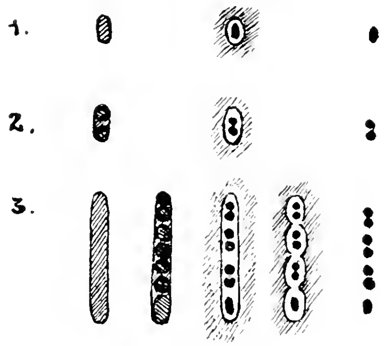
We purpose, if possible, making a series of observations upon those conditions which lead to the taking up of bacteria from the intestines and their course through the blood and again through the lymphatic system.

Finally, a few words regarding the structure of the colon bacillus to be deduced from the above observations. It is difficult to arrive at any other conclusion than that the type of this organism is a stumpy bacterium with rounded ends. This bacillus consists of at least two parts, one which takes deeply the stain, the other relatively non-staining. Under ordinary conditions of free and rapid growth, these are not to be distinguished from each other—under other conditions, more especially those of difficult growth, the chromatin or staining portion tends to be aggregated along the axis of the bacillus, most characteristically in the stumpy bacillary form as two rounded bodies—and thus the appearance is given of a diplococcus, a capsulated diplococcus, the apparent capsule being the non-staining body substance.

Where the bacillus is of the large or filamentous type, our observations would seem to show us that the filament is capable of being broken up with a certain amount of ease, *e. g.* in the endothelial cells, into its component stumpy or bacterial forms: each of these being either a single oval deeply-staining body, or, as above-mentioned, two rounded staining bodies, so that it assumes the diplococcus form. The size of these chromatin bodies varies as would be the case were the chromatin capable of varying degrees of concentration.

Judging from what is observed within the hepatic and renal cells, these chromatin bodies consist at least of a basal material or framework and a chromatin: for the power of staining (with aniline dyes) may completely disappear and nevertheless a substance is left behind still capable of recognition as a minute diplococcus, unstained by ordinary reagents but within the liver cell capable of taking up a brownish pigment. From the appearance within the body cells this central substance is obviously more resistant than the remainder of the bacillus. It is the last part of the bacillus to be destroyed—indeed, these diplococcus-like shadows of bacilli may accumulate within the liver cells more especially, and the mesenteric and retroperitoneal glands.

We will not here discuss the recent work upon the existence of nuclei or of nuclear material in the schizomycetes. It is, however, impossible not to be struck by the analogy in structure between the colon bacillus as here described and nucleated cells in which nuclear division precedes cell division. For we have the forms shown in the accompanying figure.



To show relationships of bacteria, ovoids and diplococcoids.

1.—Variations in stumpy bacterial form.
2.— " " form with polar staining.
3.— " filamentous form.

This paper being already longer than we had intended to present we have omitted any consideration or criticism of the observations by Gärtner (8), Klein (9), Thiercelin (10) and many others, which show a recognition or failure of recognition of the existence of this diplococcoid form of the colon bacillus, and very frequently a tendency to mistake the diplococcoid and encapsulated form of the colon bacillus for an entirely different species. This subject will be discussed at greater length by one of us (M. E. A.) in a separate article.

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DESCRIPTION OF PLATES XVIII-XX.

Plate XVIII.

The figures of bacteria have all been drawn (by J. G. A.) under the same magnification, *i. e.* Reichert $\frac{1}{18}$ in. immersion, ocular 4, by means of a Zeiss camera lucida, latest pattern. All were subjected to the same process of staining: Ziehl-Neelsen carbol-fuchsin diluted with 50 per cent. of water. They were relatively deeply stained and then decolorized in water containing a minute proportion of acetic acid—about one drop of glacial acetic acid to the ounce of water. The drawings of sections were made under the same conditions, with the exception of Fig. 11, which is a tracing from a photograph.

Fig. 1. Growth originating from bile of guinea-pig which died 24 days after intraperitoneal inoculation with 10 ccm. of ascitic fluid from case of hepatic cirrhosis; 48 hours' growth in alkaline peptone broth inoculated from 48 hours' culture upon agar-agar, which in its turn had been seeded from a pipette of the guinea-pig's bile removed a few minutes after death and kept for 18 hours at 37°.

Fig. 2. From film made from the ascitic fluid of the above of hepatic cirrhosis left in sterilized flask at 37° for 17 days.

Fig. 3. From first broth culture, 48 hours old, made from the above ascitic fluid; note minute ovoids as well as diplococci.

Fig. 4. From 48-hour culture upon Löffler's blood-serum made direct from the above ascitic fluid; forms a shade larger than those from broth, with slight tendency to be arranged in short chains.

Fig. 5. The same microorganism after repeated transfer upon agar-agar during 6 months; individuals much larger, although still relatively small, with short bacillary, diplobacillary, and diplococcoid forms.

Fig. 6. The same after transfer through three guinea-pigs, followed by three successive transfers through 2.5 per cent lactose broth; bacillus now is morphologically indistinguishable from the forms seen in Fig. 1 and (save in absence of flagella) from the ordinary colon bacillus.

Fig. 7. Microbe isolated from peritoneal exudate in case of peritonitis with perihepatitis after parallel passage through three guinea-pigs and transfer through lactose broth. The microbe, upon first isolation, was a minute diplococcus. It will be seen to be indistinguishable from that shown in Fig. 6; culturally, it was identical.

Fig. 8. To show arrangement of diplococcoids and ovoids in filamentous form of a colon bacillus isolated from the spleen in a case of progressive

hepatic cirrhosis. From a colony in an agar plate kept 5 days under unfavorable conditions (vide *British Medical Journal*, October 22, 1898).

Fig. 9. From film made from the bile of a rabbit killed 7 hours after intravenous inoculation with the colon bacillus; the bile was kept 14 days in pipette before examination. Note variety of forms: rare short bacilli and diplobacilli with slight capsule, diplococci with well-marked capsules, minute diplococci (? destroyed) devoid of capsule.

Fig. 10. Cells from peritoneal fluid of guinea-pig killed 7 hours after intraperitoneal inoculation with a 48-hour broth culture of form shown in Fig. 5, *i. e.* from agar cultures derived from the ascitic fluid from a case of cirrhosis. Stained with carbol-thionin, Reichert $\frac{1}{18}$ in. immersion, ocular 4, drawn under a Zeiss camera lucida. *a.* Deeply-stained bacterial and diplococcoid forms. *b.* Attenuated diplococcoids in large vacuoles. *c.* Still further attenuated diplococcoids. *d.* Vacuoles void of contents.

Fig. 11. From section of convoluted tubules of kidney of young rabbit killed two hours after intravenous inoculation with pure culture of Bacillus coli. Tracing from photograph under Zeiss $\frac{1}{18}$ in. immersion, compared with original section (the photograph not being perfect). *a.* Deeply-staining diplococcoid form just within cell of tubule. *b.* Attenuated diplococcoid form in vacuole. *c.* Empty vacuoles of oval shape.

Fig. 12. To compare with Fig. 1. From film of bile from guinea-pig kept in pipette and placed in incubator at 37° for eighteen hours. A drop of this same bile passed through broth and agar gave the form shown in Fig. 1. Note presence of ovoids, diplococcoids, and diplococci.

Plate XIX.

Fig. 13. Section of liver of rabbit killed 15 minutes after intravenous inoculation with 0.5 cc. of 48-hour broth culture of B. coli; carbolic thionin: Zeiss camera lucida; $\frac{1}{18}$th oil immersion.

a. Marked swelling and enlargement of endothelial cell with ingestion of bacilli, which have become short and stumpy.

b. Free bacilli, remaining long.

c. Leucocytes containing bacilli.

Fig. 14. Section of liver of rabbit killed 15 minutes after intravenous inoculation with 0.5 cc. of 48-hour culture of B. coli: carbolic thionin: Zeiss camera lucida; $\frac{1}{18}$th oil immersion lens.

a. Bacillus free in capillary.

b. Endothelial cell, much swollen and containing several bacilli, both stumpy and tending to assume diplococcoid form.

Plate XX.

Fig. 15. Section of liver of rabbit killed 4 hours after intravenous inoculation with 0.5 cc. of 48-hour culture of B. coli; carbolic thionin; Zeiss camera lucida: $\frac{1}{18}$th immersion lens.

Abundant very minute diplococcoid forms in liver cells, part of which only are shown.


1
2
3
4
5
6
12
7
8
9
10
11
a
b
c
d

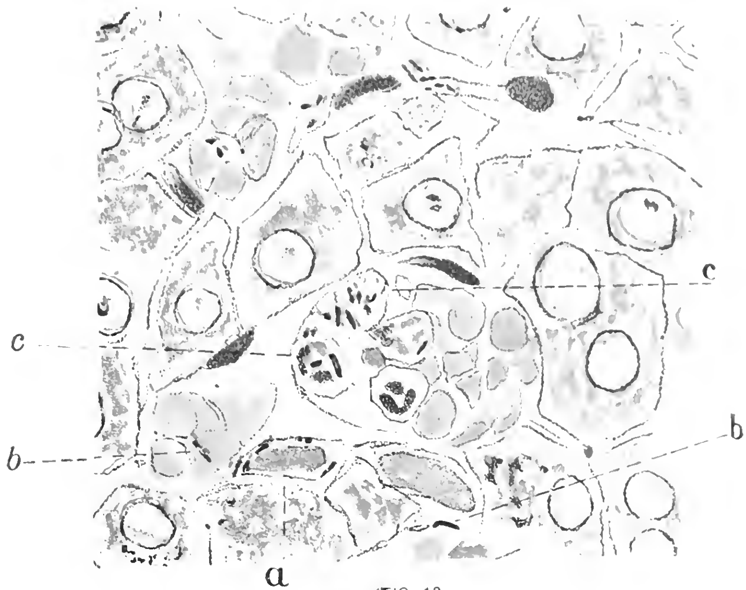

FIG. 13.

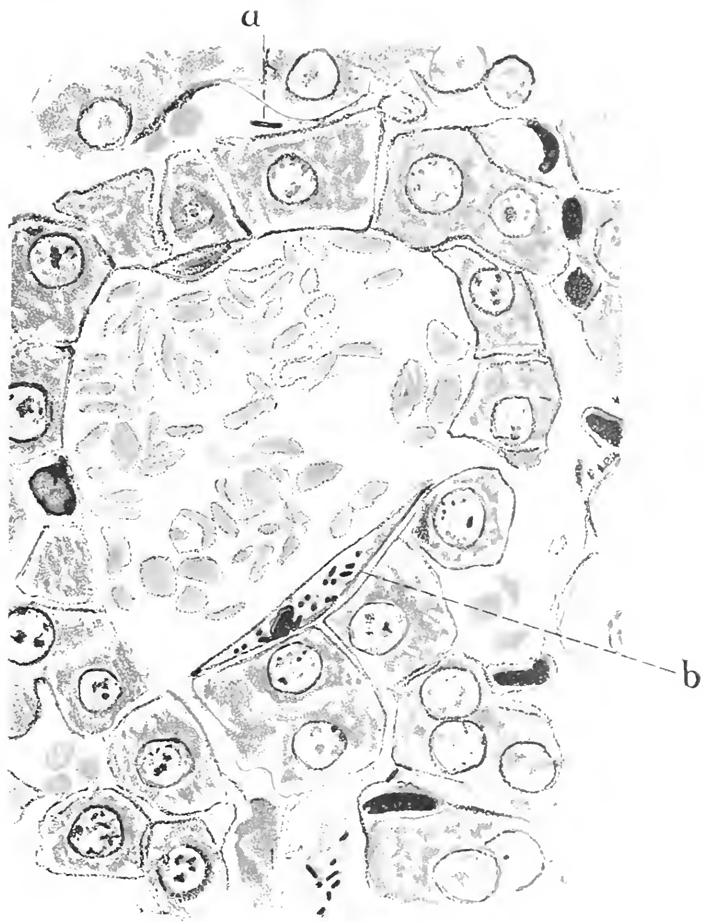

FIG. 14.

FIG. 15.

THE RELATION OF DEXTROSE TO THE PRODUCTION OF TOXIN IN BOUILLON CULTURES OF THE DIPHTHERIA BACILLUS.

By THEOBALD SMITH, M. D.

(*From the Pathological Laboratory of the Massachusetts State Board of Health.*)

The publication of certain investigations by Spronck and van Furenhout * in 1895 concerning the inhibitory action of muscle sugar upon the production or accumulation of toxin in peptone bouillon cultures of the diphtheria bacillus was the starting point of a series of investigations into this practically very important subject. Before the publication of this paper I had pursued a similar line of observations. The method employed was to test the amount of muscle sugar present in beef bouillon in the fermentation tube and compare it with the relative toxin production. It was found that the least amount of sugar was associated with the largest accumulation of toxin. Owing to the scarcity of beef containing but traces of sugar the work progressed slowly and did not appear until 1896.† In the meantime Park and Williams ‡ had found that so far as the beef used by them was concerned, the inhibitory action of the muscle sugar could be neutralized by making the bouillon sufficiently alkaline. Cobbett § in a later paper confirms the relations between muscle sugar and toxin production. Blumenthal|| reports upon the use of large quantities of sugar (grape and milk sugar) in cultures of the diphtheria bacillus. His results are wholly unintelligible to me. Among other things he states that lactose is acted upon by diphtheria bacilli, whereas I find that the addition of lactose does not influence the culture whatever. He also

Annal. de l'Inst. Pasteur, 1895, ix, p. 758.

† *Trans. Assoc. American Physicians*, 1896, xi, p. 37.

‡ *Journal of Experimental Medicine*, 1896, i, p. 164.

§ *Annal. de l'Inst. Pasteur*, 1897, xi, p. 251.

|| *Deutsche med. Wochenschr.*, 1897, p. 382.

states that sugar bouillon inoculated with diphtheria bacilli becomes "very frequently" acid. This depends upon the bouillon, whether free from muscle sugar or not, and the kinds of sugar added. The chemical changes under like conditions are absolutely constant. Blumenthal goes so far as to vindicate a therapeutic action for sugar because of its supposed inhibitory action on toxin production, a very premature inference, as this paper will show.

Madsen * in an otherwise interesting paper presents nothing new concerning the factors favoring or opposing toxin production.

More recently Martin and Spronck have published methods by which they claim to have produced unusually toxic culture fluids. Martin† prepares his peptones by the self-digestion of the stomachs of swine. The resulting fluid is added to an equal quantity of fermented bouillon. The mixture is heated to 70° C. and then passed through a Pasteur filter. If Martin's method should prove to yield all that is claimed for it, it would be superior to any now in use. A recent careful trial has convinced me that it is liable to fail in producing the looked-for result. I obtained from bouillon prepared in this way a filtrate having from $\frac{1}{5}$ to $\frac{1}{8}$ the toxic power of the filtrate obtained according to the method given below. The fermentation tube revealed the presence of a considerable amount of sugar. Whether this was the cause of the failure I am not prepared to state. That the method may under certain circumstances accomplish all that is claimed for it I will not gainsay, but it does not appear to act uniformly and the results cannot be predicted as with the method to be described. Spronck's ‡ new method utilizes, in place of beef juice, the boiled and filtered extract of the yeast of commerce to which he adds salt and 2 per cent peptone. This fluid yields a toxin 20 times stronger than does the bouillon from decomposed beef, the minimum fatal dose for a 500-gramme guinea-pig being now .005 cc.

The process which I wish to describe is a slow evolution of the past three years. Owing to the necessity of keeping on hand large quantities of diphtheria toxin for practical purposes, the investigations could

* *Zeitschr. f. Hygiene u. Infectionskrankheiten*, 1897, xxvi, p. 157.

† *Annal. de l'Inst. Pasteur*, 1898, xii, p. 26.

‡ *Annales de l'Inst. Pasteur*, 1898, xii, p. 700.

not be pushed rapidly and I contented myself with gradually introducing modifications and carefully noting results. The process cannot be considered essentially new excepting in so far as the minor details here added are absolutely necessary to its success. In the course of the work it was found, contrary to all the views hitherto expressed, *that dextrose is not in itself inimical to toxin production, that a certain quantity is in fact essential to an abundant accumulation of toxin.* It was found that the muscle sugar naturally present in beef and the ordinary chemically pure dextrose, added after the former had been removed by fermentation, act in a quite different manner and that we must assume either that the muscle sugar undergoes a decomposition under the influence of the diphtheria bacillus different from that which ordinary dextrose undergoes, or else that there are other still unknown inhibitory substances in the beef which are removed with the muscle sugar during the preliminary fermentation. Leaving a discussion of the experiments which demonstrated this peculiar behavior of unfermented bouillon aside for the present I will give a description of the process as at present in use.

PREPARATION OF PEPTONE BOUILLON FOR TOXIN PRODUCTION.

It should be stated at the outset that now and then bouillon containing a little muscle sugar will yield a toxin as strong as bouillon specially prepared. This outcome cannot be predicted however. Following the suggestions of Park and Williams that the difficulty can be overcome by the increased alkalinity of the bouillon I have returned again and again to unfermented bouillon without obtaining so good results as with the new method. Until the beef used in different localities has been compared, these discrepancies cannot be explained satisfactorily.

1. The beef infusion is prepared in the usual way and kept in the cold for 12 to 24 hours. The beef juice is then expressed, and its reaction, which will in general be found to vary from 3 to 4 per cent acidity,* must be reduced to 1.5 to 2 per cent by the addition of normal sodium carbonate solution. The fluid is then heated to 40° C., inoculated with

* See below for a definition of these terms.

30-50 cc. of a 12- to 24-hour bouillon culture of *B. coli* and placed in the incubator for 16 hours or over night. Next morning the acidity will have risen again to 3-3.5 per cent and a scum may have formed on the surface. The odor varies and may be distinctly sour or slightly putrefactive.

2. The fermented infusion is next mixed with the white of egg in the proportion of one egg to a litre of infusion, and boiled in a water-bath or an Arnold sterilizer for 45 to 60 minutes.

3. The boiled infusion, previously cooled off to favor any precipitation, is filtered and then receives 2 per cent Witte peptone, 0.5 per cent common salt, and after these have been dissolved by gentle heat, enough normal sodium carbonate solution to bring the acidity down to about 0.8 per cent. The fluid is boiled or steamed again for 20 or 30 minutes and then filtered. The reaction may become slightly more acid than the calculation allows but no further addition of alkali is necessary.

4. The filtered fluid is distributed into Fernbach flasks in shallow layers 2.5 ctm. deep and autoclaved (at 110° to 115° C. for about 30 minutes).* Each flask should have 2 or 3 cotton-plugged openings to facilitate ventilation.

5. Before inoculation with the diphtheria bacillus 5 cc. of a sterile 20 per cent solution of dextrose or about 0.1 per cent (autoclaved and kept on hand in small tubes) is added per litre.†

6. The culture employed should form membranes promptly and leave the fluid clear. This property can be induced in freshly isolated cultures by 5 to 10 transfers in bouillon of the kind here described. These can be made in large test tubes kept in an inclined position to increase the surface area.

7. The culture fluid becomes distinctly alkaline to phenolphthalein in from 6 to 8 days and may then be regarded at its maximum toxicity.

Before proceeding to a discussion of the more essential points—the relation of dextrose and peptone to the accumulation of toxin—a brief explanation of the minor details of this method will be in order.

* The necessity for autoclave sterilization was pointed out by me in *Journal of Experimental Medicine*, 1898, iii, p. 647.

† Several trials have shown that the efficiency of the bouillon is not impaired by adding the dextrose before the final autoclaving. This would materially simplify the work and reduce the chances of contamination. The amount of sugar has been increased by me to .15 and even to .2 per cent without interfering with rapid alkali production. In some instances the toxin was markedly increased, in no instance reduced in amount. For different bacilli the most favorable quantity should be determined by trials.

The process of preparing dextrose-free bouillon was first described by me in 1897.* Subsequently Martin† used the same process but substituted yeast, or left the fermentation to be accomplished by the miscellaneous bacteria already in the infusion.‡ The first method suggested by Spronck I found inadequate. The bouillon prepared from the old beef frequently contained large quantities of fermentable substance and was never entirely free from it. The process here suggested produces a bouillon which permits no growth whatever in the closed branch of the fermentation tube. It is in fact almost wholly free from reducing substances. While the ordinary bouillon, even when gas is not produced in it, still contains enough reducible substances to decolorize methylene-blue in the fermentation tube over night, the bouillon thus prepared does so only after 3 or 4 days in the incubator.§ In order to obtain this result, however, it is necessary to reduce the initial acidity of the raw infusion as directed, otherwise the additional acid formed during the fermentation may become inhibitory. It is also necessary to warm the infusion if large quantities are prepared, otherwise 3 or 4 hours will be lost and the infusion may still contain acid-forming substances next morning. The fermented infusion even after prolonged boiling forms such a loose clot that the filtration may become exceedingly tedious. To obviate this, egg-albumen should be added. Careful tests showed that the dextrose added in the egg-albumen cannot be recognized in the finished bouillon and is therefore a negligible quantity. The other parts of the process need no special explanation. The initial acidity recommended has been found the best level from which to start. The reason for the final addition of about 0.1 per cent dextrose will be given farther on.

With this method in use there has been no noticeable fluctuation in the toxic strength of the culture fluid after 6 to 8 days' incubation. The end reactions are in all cases absolutely the same excepting in flasks accidentally contaminated. In fact the production of toxin has

* *Journal of Experimental Medicine*, 1897, ii, p. 543, and 24th Annual Report of the State Board of Health of Mass. (a comparative study of the toxin production of diphtheria bacilli) issued October, 1897.

† Loc. cit.

‡ It might be supposed that the fermentation would lead to the production of various toxins. Repeated injection of 5 cc. of the finished product into the peritoneal cavity of guinea-pigs had no effect whatever.

§ Th. Smith, Reduktionserscheinungen bei Bakterien, etc., *Centralbl. f. Bakt.*, 1896, xix, p. 181.

been brought to the level of a chemical process. Formerly, the peptone was frequently suspected of being at fault, but the uniform results now obtained indicate that this suspicion was unfounded. The culture employed in these investigations, with the exception of certain final tests to be described farther on, is the one used by Park and Williams in their investigations and denominated by them "No. 8." The efficiency of the method is most convincingly shown by a record of the toxic strength of the culture filtrate. Park and Williams in their article state that .005 cc. of their strongest toxin proved fatal to a 500-gramme guinea-pig in 3 days. Martin states that the m. f. d.* for a 500-gramme guinea-pig of the strongest toxin he obtained with the same bacillus was .002 cc. In another place he mentions the dose of .005 cc. for a 500-gramme pig. The following consecutive record of diphtheria toxins prepared according to the procedure described shows the unvarying results obtainable. The test was made in all cases from mixtures of about 4 litres each of filtered culture fluid, either immediately after filtration or some months later:

Lot	1.	.01 cc.	fatal to	318-gramme	pig	in	28	hours.		
"	2.	.01 cc.	"	362	"	"	36	"	±
		.0035 cc.	"	257	"	"	"	"	±
		.0025	"	253	"	"	60	"	±
		.0023	"	256	"	"	$5\frac{1}{2}$ days.		
"	3.	.01 cc.	"	322	"	"	36	hours	±
"	4.	"	"	276	"	"	36	"	+
"	5.	.01 cc.	"	300	"	"	28	"	
		"	"	"	"	"	36	"	±
		.005	"	309	"	"	40	"	
"	6.	.01	"	376	"	"	"	"	±
		.005	"	432	"	"	48	"	—
"	7.	.01	"	356	"	"	24	"	
		.005	"	312	"	"	36	"	±
"	8.	.01	"	360	"	"	"	"	±
		.005	"	378	"	"	54	"	
"	9.	.005 cc.	"	371	"	"	36	"	±

* Abbreviation for minimum fatal dose.

Lot 1.0	.01 cc.	fatal to	405-gramme	pig	in	57 hours.	
" 11.	"	"	390	"	"	5¼ days.	
" 12.	"	"	250	"	"	36 hours	±
" 13.	"	"	378	"	"	30 "	±
	"	"	383	"	"	" "	±
" 14.	"	"	365	"	"	54 "	±
	"	"	365	"	"	56 "	±
" 15.	"	"	442	"	"	60 "	±

In Lots 1 and 2 the 2 per cent peptone was added from a sterile solution just before inoculation. In lot 13 there was still some muscle sugar. In lot 11 the dextrose was added *before* the final autoclaving.

In estimating the absolute toxicity of culture filtrates the relative susceptibility of the guinea-pigs used must be taken into consideration. Animals from some sources seem to be much more susceptible to diphtheria toxin than those from others. For several years the writer had been experimenting only upon guinea-pigs reared under his supervision. During this time all animals used exhibited a remarkably uniform susceptibility. Latterly guinea-pigs purchased from a dealer had to be used and it was soon evident that for them the m. f. d. was about $\frac{1}{2}$ to $\frac{3}{5}$ of that to which the home-bred pigs succumbed. Similar differences were noticed when toxin-antitoxin mixtures in which there was a slight excess of toxin were injected. Many of the animals used by the writer for breeding purposes had passed through a single inoculation with toxin or toxin plus antitoxin at least 3 or 4 months previously. Recently Behring * states that he uses such guinea-pigs in the same way but has not noticed any increased resistance in the progeny. He states furthermore that Ehrlich obtained from a breeder a race of diphtheria-immune guinea-pigs and that in England these animals present a considerable degree of resistance to diphtheria toxin. The more susceptible animals used by the writer were as a rule thin and had a thin skin, while those raised for the laboratory had a thicker skin and were in excellent condition. This possible variation in the resistance to the diphtheria toxin must first be taken into account before we can positively decide which method may give the strongest

* *Deutsche med. Wochenschr.*, 1898, p. 621.

toxin. However, with the figures quoted above and those to follow as a basis there seems little to choose between Martin's complicated and not certain method and the simple one I have described.

THE RELATION OF DEXTROSE TO THE REACTION CURVE OF PEPTONE BOUILLON AND TO TOXIN PRODUCTION.

The daily changes in the reaction of the culture fluid are perhaps the best available indications of the activity of the bacilli and of the toxin production. In order to follow this change closely without disturbing the surface membrane which forms within 24 hours the following procedure was adopted:

A large Fernbach flask* (Figure 1, reduced to $\frac{1}{3}$ size) within which a litre of bouillon occupies a layer 2.5 ctm. deep, is modified so as to have 3 cotton-plugged openings. Through one lateral opening a siphon (*A*) passes which has joined by means of rubber tubing to its lower free end a protected mouth-piece according to Maassen (*B*). The other lateral opening may have in it a small funnel (*C*) through which alkalies, acids or other fluids may be added without disturbing the larger plug or breaking the surface membrane. The three openings are also very favorable to free ventilation. The flask after inoculation is placed on a shelf in the thermostat so that the longer arm of the siphon may pass through a hole in the shelf. From day to day or oftener if desired fluid may be removed for various tests without disturbing the flask or imperiling the purity of the contents. Care should be taken to reject the fluid in the siphon as it has been under anaërobic conditions since the former withdrawal of fluid. The samples thus obtained were titrated with phenolphthalein as an indicator according to the method suggested by Fuller† and the values obtained are expressed throughout this article in per cent of a normal solution of acid or alkali. The sign minus (—) whenever used signifies acid, the sign plus (+) alkaline reaction toward phenolphthalein.‡

* Made for the writer by Whitall, Tatum & Co., N. Y.

† Procedures recommended for the study of bacteria, Concord, N. H., 1898, p. 19. See also *Journ. Amer. Public Health Assoc.*, 1895, p. 386.

‡ This use of the signs is contrary to the notation adopted by the committee which edited the "Procedures," etc., and of which the writer was a member. My reason for the use of + for alkalinity is that all aërobic bacteria, both obligatory and facultative, tend normally towards an alkaline reaction. The tendency towards an acid reaction is in a sense abnormal and, if continued, destroys the organisms producing it. This notation was adopted after careful deliberation as being more logical.

The presence or absence of sugar in the finished bouillon was determined with the aid of the fermentation tube and *B. coli.* The rise in acidity of the fluid in the closed branch, whether gas appears or not, is an indication of the presence of dextrose or allied substances (excluding glycogen). The amount of acid produced is proportional to the amount

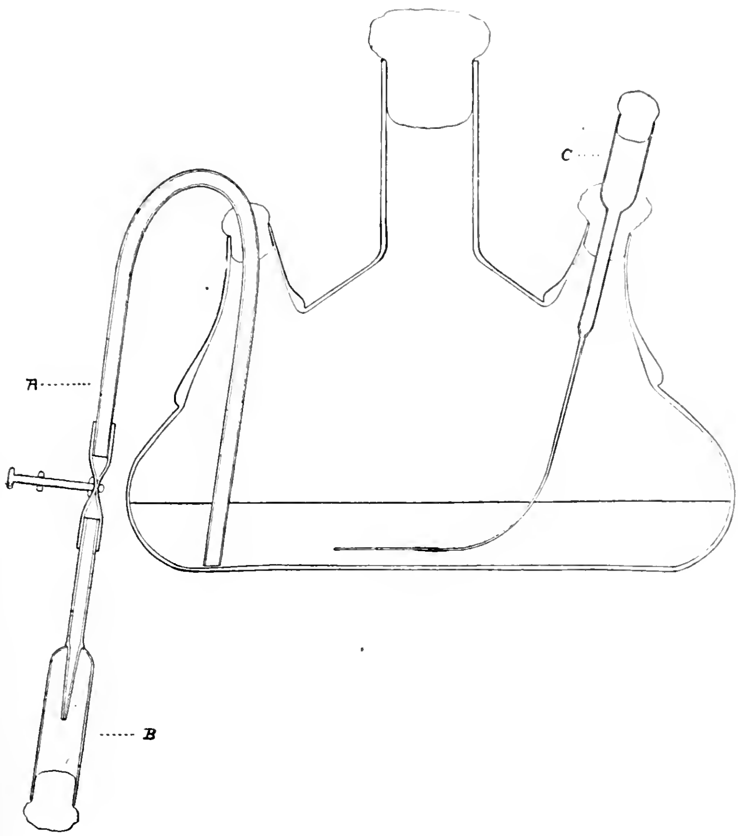

Fig. 1.

Fernbach Flask, modified by the writer. Figure reduced to $1/3$ size.

of sugar present up to a certain limit varying for different bacteria and probably never exceeded by the sugar in beef broth. A total absence of sugar is indicated by an absence of growth in the closed branch. *B. coli* (as well as other facultative anaërobes) becoming obligatory aërobes when sugar is absent.

As stated in the introduction, it has been generally assumed that the amount of acid formed by diphtheria bacilli in presence of muscle sugar is responsible for the feeble toxin production. In the course of these studies I was early convinced that these bacilli in their multiplication can without trouble produce and neutralize much larger quantities of acid derived from dextrose, artificially added, than are as a rule formed in unfermented bouillon. In order to attempt an explanation of this paradox, it became necessary to learn what strength of acid is injurious to the toxin. As a preliminary test dextrose was added to a full-grown alkaline culture to see what effect the acids, produced by the diphtheria bacillus itself, had on its toxin.

I. February 5, 1897. To a 10-day litre culture with reaction + 0.2 (*i. e.* nearly as strong an alkaline reaction as such cultures can attain) of which .01 cc. is fatal to a 340-gramme guinea-pig in 2½ days, enough sterile dextrose solution is added to make a one per cent solution.

February 7. A good membrane has formed in place of the former one shaken down February 5.

February 10, reaction—4.77.

" 13, 0.03 cc. produces no longer any local effect.

" 17, 0.5 cc. " " " " " "

" 18, reaction as on February 10, no change.

" 23, one cc. of this culture, which had been filtered and stored in the cold carefully neutralized with NaHO has no effect on a guinea-pig.

II. February 17, 1897. To a 22-day culture of another diphtheria bacillus of which the m. f. d. is now 0.04 cc. one per cent dextrose is added.

February 18. Renewed multiplication.

" 19. Complete membrane.

" 23. Reaction. —4.55; 0.5 cc. has no effect on a guinea-pig.

" 27. Subculture remains sterile.*

These experiments show that with the two cultures tested the maximum acidity does not exceed 4.5 to 5 per cent. 5 or 6 days after the beginning of renewed growth and acid formation the toxin present at the start is completely destroyed. Later the bacilli themselves are killed.

* Cobbett (l. c.) states that diphtheria bacilli are *not* killed by the acids they produce, while colon and other bacilli are so destroyed.

It now became necessary to determine the effect of different degrees of acidity within the maximum. This could be most expeditiously done by adding to the finished and filtered toxin certain acids in known quantities. For this purpose lactic acid and chlorhydric acid were chosen. The toxin employed contained about 0.2 per cent carbolic acid. The acidified toxin was kept in partly-filled, cork-stoppered bottles in the thermostat to imitate as nearly as possible the usual conditions of the culture.

I. M. f. d. of toxin about 0.04 cc. Lactic acid added to an acidity of 2 per cent. After 1, 6 and 13 days no appreciable loss in toxicity.

II. M. f. d. of toxin 0.036 cc. Lactic acid added to — 4.4 per cent. After 24 hours .08 cc. produced only local necrosis. After 3 days 0.3 cc. no longer fatal. After 6 days 1 cc. produces only transitory œdema.

III. The same toxin (m. f. d. = .036 cc.) receives chlorhydric acid to — 4.47 per cent. After 6 days 1 cc. produced severe necrosis locally.

IV. This and the following test were made a year later. M. f. d. of toxin .032 cc. Lactic acid was added to produce reactions of — 3.5, — 4, — 4.5 and — 5 per cent. Actual acidity found to be — 3.66, — 4.15, — 4.65, — 5.12 per cent.

After 5 days none of the 4 acidified toxins produced any local lesion in doses of 0.064 cc. After 11 days 0.5 cc. of lowest acid toxin (— 3.66 per cent) had no effect.

V. The same toxin brought to an acidity of 2.8 and 3.2 per cent with lactic acid was tested after 5 and 9 days. The m. f. d. of the first toxin after 5 days was about .045 cc., after 9 days 0.1 cc. The second toxin produced only a slight local effect in a dose of 0.06 cc. after 5 days. After 9 days 1 cc. still produced local necrosis.

The m. f. d. of the control toxin rose in 5 days from .032 cc. to .045 cc.; in 9 days to .06 cc.*

These tests though incomplete in many respects indicate that an acid reaction of 2.5 to 3 per cent destroys the toxin only very slowly, while above 3 per cent the destruction is more rapid. A reaction of

* The destruction of toxin in cultures provided with protecting bacillar membranes does not proceed so rapidly as this in the thermostat. From an earlier experiment the following figures may be quoted:

After	8	days of growth	0.02	cc. fatal	to a	410	gramme	guinea-pig in	2 days.
"	34	"	0.02	"	"	435	"	"	3 "
"	56	"	0.04	"	"	305	"	"	3½ "

— 3.5 per cent or above is quite rapidly destructive. The maximum amount of acid produced by most diphtheria bacilli (4.5 to 5 per cent),

Fig. 2.

which is destructive to both toxin and bacilli, is equivalent to .164 to .182 per cent pure chlorhydric acid.

The quantitative production of acids in ordinary, unfermented

bouillon varies considerably, but it rarely rises above 3 per cent if the initial acid reaction is fairly low (0.8 to 1 per cent). It is difficult,

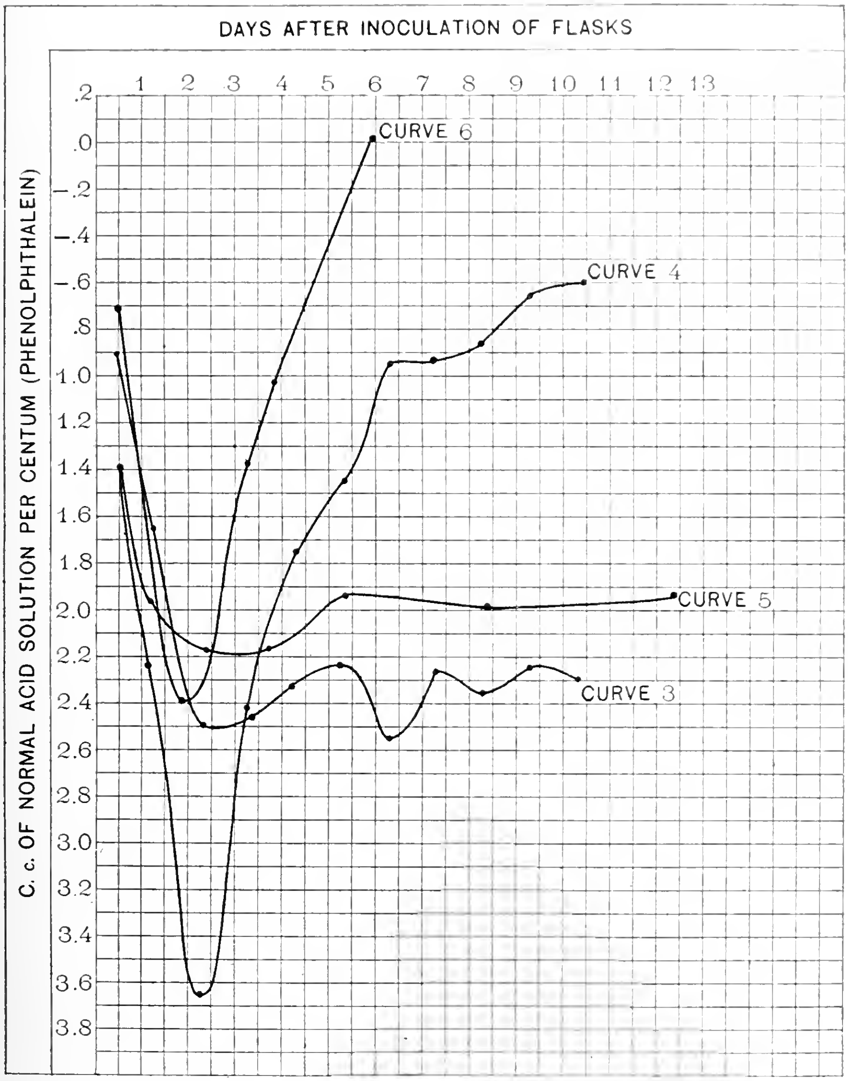

Fig. 3.

therefore, to harmonize the inhibitory power of these acids, if it really exists, with the figures quoted above—that an acidity of 2.5 to 3 per

cent is only slowly destructive. On the other hand a higher temporary degree of acidity is compatible with an abundant accumulation of toxin when the acids are derived from dextrose added. From among the many experiments made the following are selected as illustrating the rapid acid production in presence of both muscle sugar and dextrose, the rapid alkali production in presence of large quantities of the latter, and the marked difference in the final accumulation of toxin in bouillon containing muscle sugar and in that containing only ordinary dextrose:

June 27, 1897. 1200 cc. of peptone bouillon containing still a very small quantity of muscle sugar (about .02 per cent) receives 18 cc. of a sterile 20 per cent solution of dextrose, or about 0.3 per cent.

June	28.	Initial acidity 1 per cent. Inoculated with diphtheria bacilli.	
"	29.	8.30 A. M.	Membrane present; acidity 2.23; 10 cc. normal NaHO added.
"	29.	5.30 P. M.	Acidity 2.16; 10 cc. NaHO again added.
"	30.	8.30 A. M.	Acidity 3.23; 10 cc. NaHO again added.
"	30.	4.30 P. M.	Acidity 2.22.
July	1.	9 A. M.	" 2.16.
"	1.	5.30 P. M.	" 1.62.
"	2.	9.40 A. M.	" 1.46.
"	3.	9 A. M.	" 0.38.
"	4.	9 A. M.	" 0.00.
"	6.	3 P. M.	Alkalinity 0.3. Culture pure; 0.04 cc. fatal to a 300-gramme guinea-pig in 36 hours.
"	14.	0.04 cc. fatal to a 313-gramme guinea-pig in 36 hours.*	

At this time the best toxin obtained from this bacillus, *i. e.* before the present method had been perfected was about .015 cc. for the m. f. d. for a 300-gramme guinea-pig. The above inoculations indicate a m. f. d. of .02 cc. The curve of this culture is platted as Curve 1, Fig. 2, in which the dotted line indicates the reduction in acidity

* In this experiment the alkali was added to prevent the acidity from ascending to the inhibitory and destructive limit. The experiment was primarily conceived to determine whether the bacterial metabolism in the presence of large quantities of dextrose would be inimical to toxin production.

brought about by the added alkali. The most favorable condition encountered with ordinary unfermented bouillon is shown by Curve 2, Fig. 2. The amount of muscle sugar was about 0.15 per cent. The reaction returned quite promptly to the neutral point and the toxicity of the bouillon was about .02 cc. But this course is not to be anticipated and may in fact be exceptional. Curve 3, Fig. 3, has been a more common type with beef used in this laboratory. The bouillon contained 1 per cent peptone and about 0.15 per cent muscle sugar. The initial acidity was reduced with alkali to 0.8. After inoculation the acidity rose to 2.45 where it remained for 14 days. On the 17th day the toxicity was about 0.09 cc.

Curve 4, Fig. 3, represents the course of a culture in dextrose-free bouillon containing 2 per cent peptone and about 0.18 per cent dextrose added before inoculation. On the 3d day the acidity had risen to 3.6. On the 5th day, when the acidity was still 1.44, the toxicity was 0.015 cc. On the 10th day it was 0.01 cc.

The following parallel tests with bouillon from the same beef, one lot fermented with *B. coli*, the other not, are still more demonstrative.

I. Dextrose-free bouillon receives in sterile solutions 1 per cent peptone and 0.6 per cent dextrose. Initial reaction — 1.4. After 7 days, reaction feebly alkaline to phenolphthalein, m. f. d. about .008 cc.

II. The unfermented bouillon containing about 0.1 per cent muscle sugar receives 1 per cent peptone. The initial reaction is — 1.4. After inoculation the acidity rises to — 2.13 and remains there for 20 days (Curve 5, Fig. 3). Toxicity at this time about 0.06 cc.* It should be noted that the bouillon used contained only one per cent peptone.

From these few illustrations among many it is evident that the amount of acid formed in ordinary peptone bouillon is not sufficient to account for the marked interference with growth, for when dextrose is added to fermented bouillon the acidity may be much greater during the first 2 days as illustrated by Curve 6, Fig. 3. There is, how-

* The inoculations were as follows:

I. 0.01 cc. fatal to 289-gramme guinea-pig in 44 hours.

II. 0.05 cc. produces local necrosis in a 289-gramme guinea-pig. After 17 days guinea-pig weighs 355 grammes.

ever, a prompt production of alkali which makes the curve of these cultures quite acute in outline. The cultures in unfermented bouillon usually languish at a comparatively low degree of acidity and the accumulation of toxin is correspondingly light.

In searching for the cause of this peculiar inhibition of the growth of the diphtheria bacillus in ordinary bouillon it occurred to me that possibly the glycogen of the muscular tissue might be responsible. This factor however was eliminated by a single experiment. The presence of glycogen had no effect upon the reaction curve of diphtheria cultures. The cultures proceeded as in dextrose-free fermented bouillon. In other words, diphtheria bacilli do not attack glycogen as they do dextrose.

Very many experiments have been made during the past two years to determine the influence of adding peptone and alkali before and after the first boiling of the beef infusion, also the behavior of peptone added before the final autoclaving and after it in sterile solution. It was thought that possibly the interaction of the different substances in presence of carbohydrates or a slight excess of either acid or alkali might produce modifications of the peptones or other substances sufficient to influence the production of toxins favorably or unfavorably. These experiments were made with the same beef infusion, each set of flasks having their contents modified in some way. Without going into detail concerning these tedious trials, it may be stated that if a bouillon gives rise to much toxin when prepared according to one method, it will yield equally goods results whatever be the order of neutralizing or adding the various ingredients. With reference to the addition of dextrose I may state that it largely disappears when added to the raw infusion. When added to the boiled and filtered infusion it is not lost.

Bouillon may even remain markedly acid during the preparation without losing its toxin-producing capacity, provided the acidity be properly reduced before use. In one instance the final acidity through some oversight was left at 3 per cent. After a reduction to 0.7 per cent with 23 cc. normal soda solution per litre and the addition of 0.12 per cent dextrose in sterile solution the fluid was inoculated. After 6 days the toxicity

was .007 cc. The course of the reaction is shown by Curve 6, Fig. 3. A duplicate flask yielded the same toxin.*

The unusually good results obtained with the dextrose-free peptone bouillon might reasonably raise the query whether the miscellaneous bacteria, including *B. coli* in the beef infusion, may not produce some substance from which toxin is easily obtained by diphtheria bacilli. It has already been stated that the infusion is considerably altered during the fermentation since the coagulation formed by boiling fails to cohere as in fresh infusion and renders filtration very difficult. Bouillon prepared in this way without peptone and tested parallel with that to which 2 per cent peptone had been added produced barely 1 per cent of the toxin found in the peptonized fluid, *i. e.* while .01 cc. or less of the peptonized bouillon proved fatal to guinea-pigs, 1 cc. of the peptone-free bouillon contained only a trace of toxin in spite of good growth, membrane formation, and final alkalinity. Nor does such bouillon give rise to any indol when indol-producing bacteria have multiplied in it. We are, therefore, justified in concluding that the fermentation does not yield any substance available for toxin production, but simply eliminates some inhibiting substance from the bouillon while the true source of the toxin is the peptone added to it.

OTHER FACTORS MODIFYING TOXIN PRODUCTION.

Besides the presence or absence of muscle sugar as a factor in the production of diphtheria toxin, there are several others which have a

* In one experiment made recently the fermented bouillon was simply left acid and without dextrose. The object was to obtain thereby the same amplitude or range of reaction otherwise secured by making the bouillon more alkaline and adding dextose which furnishes the acids. The result was as good as when the latter method is employed. Thus a flask of 2 per cent peptone bouillon with an initial reaction of — 2.2 yielded on the 9th day an alkaline fluid whose m. f. d. was about 0.004 cc. (compare with Table I. p. 391, with which this test was made). Whether this procedure would be always successful and capable of taking the place of the alkali plus dextrose can only be decided after repeated trials. Another modification of the process consisted in the fermentation of the boiled and filtered broth instead of the raw infusion. The peptone was added after the bacteria (B. coli and others) had been eliminated by boiling and filtration. This modification, tried but once, also yielded a strong toxin.

distinct influence and which, differently employed by different observers, prevent any very accurate comparison of published results. Among these the most important are:

1. The amount of peptone used.
2. The manner in which the stock cultures have been kept.
3. The oxygen supply (character of the culture flask and the depth of the layer of fluid).

Other possible modifying influences, such as the method of preparing and sterilizing the bouillon and the initial reaction do not in my experience have any marked influence so long as the reaction attained by the culture does not reach the inhibitory limit above — 3.5 per cent.

The amount of peptone.—It is a well-known fact that of the peptones added to culture media only a small amount is utilized by bacteria. For a number of years the writer used only ¼ per cent peptone for culture media. The return to one per cent was simply to conform to current methods. In the production of diphtheria toxin 2 per cent has been used by some, 1 per cent by others. A number of special trials have been made in combination with the present method of preparing the bouillon, to determine the relative efficiency of different amounts of peptone.

The same dextrose-free bouillon, placed in thin layers, 2-2.5 cm. deep, in Erlenmeyer flasks, receives (*a*) 0.5, (*b*) 1, and (*c*) 1.5 per cent peptone. The initial reaction is — 0.87, — 0.91, and —0.8 respectively. Each flask receives 0.1 per cent dextrose and is then autoclaved. On the ninth day after inoculation the fluid is alkaline in all flasks.

(a).	.01 cc.	fatal to	240-gramme	guinea-pig	in	1½	days	±,	m. f. d.	.005 cc.
	.005 cc.	"	254	"	"	" 3¾	"			
(b).	.008 cc.	"	235	"	"	" 1½	"	±	"	.0025 cc.
	.003 cc.	"	300	"	"	" 1¾	"			
(c).	.005 cc.	"	268	"	"	" 1½	"	±	"	.0025 cc.
	.003 cc. produces induration only.									

Leaving aside the result of the last test as quite irregular, we notice the large amount of toxin produced in bouillon containing but 0.5 per cent peptone. The difference between 1 and 1.5 per cent peptone may be regarded as trifling. A second experiment was made subse-

quently in which not only the peptone but also the dextrose and the stock culture were varied. The other conditions remained the same. The cultures, which were derived from the same original stock (Park and Williams), had the following history:

α. Grown in bouillon for a number of years.

β. Grown on Löffler's (horse) serum for 9 months, before that in bouillon.

γ. Grown on serum for 18 months before that time in bouillon. β and γ were passed through two tubes of bouillon before use. The bouillon was fermented.

TABLE I.

Culture.	Peptone in per cent.	Dextrose in per cent.	Initial reaction.	Reaction (after 9 days).	Toxicity (after 9 days).	Estimated m. f. d. for 250-280-gramme guinea-pig.
α	1	0.1	−1.	+.2	.008 cc. fatal to 273-gramme guinea-pig in $1\frac{3}{4}$ days ±	.005 cc.
					.008 cc. " 255 " " " " ±	"
β	1	0.1	−1.	+.2	.008 cc. " 273 " " " 3 "	.007
α	2	0.2	−.95	+.05	.008 cc. " 284 " " " 31 hours−	.002 +
β	2	0.2	−.95	+.15	.008 cc. " 284 " " " 31 " +	.003 −
α	2	0.1	−1.	+.05	.005 cc. " 301 " " " $3\frac{3}{4}$ days	.005 −*
					.008 cc. " 280 " " " $1\frac{3}{4}$ " ±	.005
γ	2	0.1	−1.	+.15	.008 cc. " 280 " " " $1\frac{3}{4}$ "	.005

*Test on 7th day.

Table I shows that with a suitably prepared bouillon the accumulation of toxin in the presence of 1 per cent peptone may be nearly, if not quite as great, as in the presence of 2 per cent. Other tests not here described taken together with these have convinced the writer that probably 1.5 per cent peptone is as efficient as 2 per cent in bouillon prepared as herein detailed. The large amount of dextrose used up by this bacillus in presence of 2 per cent peptone and the conse-

quent increase in the toxicity of the culture fluid is well shown in the 3rd and 4th lines of the table.

The stock culture.—The favorable influence of the continued growth in bouillon is evident from the preceding experiment and deserves careful attention. Bouillon cultures of diphtheria bacilli undergo certain changes when, as first suggested by Park and Williams, the bacilli are transferred at short intervals from tube to tube. Those which I have treated in this way grow at first diffusely through the bouillon and only a very faint pellicle appears on the surface after some days. If the cultivation be continued, the membrane becomes heavier and the tendency to a diffuse clouding becomes more or less checked. If such culture be shaken up after a growth of 4 or more days and compared with one inoculated directly from serum, it will be found full of flakes and of a decidedly yellowish tinge as compared with the uniformly turbid original. The color approaches that of the bouillon because the flakes disperse the light less than the fine powdery suspension in the original culture. A tendency to cohere is developed in the bouillon, which tendency favors surface growth. This condition is favorable to toxin production, especially in bouillon made from unfermented beef. In fermented bouillon the difference is less marked. The great advantage of the latter bouillon appears when cultures are made of bacilli grown on Löffler serum and those freshly isolated from the throat whose capacity to grow on the surface is restricted. This is the only explanation that can be found at present for the many failures to obtain strong toxin some years ago when the work of preparing antitoxin was started with ordinary bouillon. Martin obtained strong toxin with his new method from fresh cultures. The two additional cultures I have tested show equally satisfactory results. Both were isolated in 1896. One of them, No. 14, of a series of 42 cultures * was the best toxin producer of the series at that time when Spronck's method of using old beef was still used. Early in 1897 the m. f. d. of a one per cent peptone-bouillon culture for a 300-gramme guinea-pig was 0.036 to 0.04 cc. In February, 1898, the test of a fresh culture yielded a m. f. d. of 0.036 cc. In

* *Twenty-fourth Ann. Rep. Mass. State Board of Health,* p. 543.

June another culture yielded a m. f. d. of 0.03 cc. From that time until October this bacillus was passed through bouillon every 4 days to improve the growth in membrane which had always been rather feeble. The toxin then produced in a bouillon containing 2 per cent peptone, filtered and stored but not tested until 4 months later, had the surprisingly low fatal dose of about 0.007 cc. The culture was then returned to Löffler's serum until March, 1899, when, after a passage through 3 tubes of bouillon it yielded a m. f. d. of 0.012 cc.

The second bacillus, No. 12 of the same series, yielded in 1896 and 1897 with Spronck's method a m. f. d. of 0.04 cc. to 0.45 cc. It has been grown continuously on Löffler's (horse) serum. In March, 1899, after a passage through 4 tubes of bouillon its toxicity was tested together with bacillus No. 14. It yielded a toxin having a m. f. d. of .02 cc. for a 260-gramme guinea-pig. More recently this figure was brought down to .015 cc. as shown in Table II below.

The influence of continuous cultivation in a favorable bouillon is illustrated in some recent tests with bacillus No. 14. Three cultures were used in the comparative test:

a. Grown as described above (about 4 months on bouillon, then about 6 months on serum, then for about 15 days on bouillon).

b. Grown for about 15 days on bouillon, before that on serum.

c. Grown for about 24 hours on bouillon, before that on serum.

The three cultures were then inoculated into fermented bouillon containing 2 per cent peptone and 0.1 per cent dextrose and the fluid tested after 10 days' growth:

a.	0.02 cc.	fatal to	255-gramme	guinea-pig	in	$1\frac{5}{6}$	days—	m. f. d.	.012.
b.	0.02 cc.	"	263	"	"	$3\frac{4}{5}$	"	" "	.02.
c.	0.02 cc.	"	267	"	"	$2\frac{3}{4}$	"	" "	.018.

The influence of the prolonged culture in bouillon had not been wiped out by the succeeding cultivation on serum in (*a*), for it produced a toxin about 50 per cent stronger than the bacillus grown on serum alone.

The marked increase in the toxicity of the bouillon cultures of these two bacilli (No. 12 and No. 14) since their isolation in 1896, is attributable in part to a doubling of the quantity of peptone, in part to the acquired power of surface growth, and in part to the thorough

removal of the muscle sugar and the addition of dextrose. The last factor evidently puts the bouillon into the most favorable condition for toxin production while the others aid in hastening it. This is of no small importance when we consider that in the thermostat there appears to be a continuous destruction of toxin going on side by side with its production. By intensifying and, therefore, shortening the life of the culture, the flasks can be removed after 6 to 10 days, according to the bacillus used, and much toxin thereby saved from destruction.

In Table II a final illustration of the relative influence of these several factors is given.

The culture used is No. 12. which had never been grown in bouillon, and which was, therefore, better adapted for this experiment than the culture of Park and Williams, whose membrane-forming power had been developed by them some years ago. The letter α stands for the continuous cultivation in bouillon through 17 transfers at intervals of 4 or 5 days, β for the same bacillus grown on Löffler's serum since its isolation until 24 hours before use when a bouillon culture was prepared. The bouillon used was deprived of its muscle-sugar by fermentation and some dextrose was added. The cultures were in Erlenmeyer flasks as for the experiment in Table I. In the α flasks after inoculation the membranes formed promptly and became heavy. The fluid remained clear. In the β flasks, on the other hand, the membranes were quite feeble and the bouillon well clouded throughout.

As a result, alkali production was much more rapid in the membrane-forming cultures than in the others, since it took the latter 15 days to reach the point probably attained by the former in 6 or 7 days. Nevertheless the accumulation of toxin did not go parallel to alkali production in the flasks containing one per cent peptone, for the toxicity was the same for the alkaline and the still acid culture on the 9th day. The relatively large amount of toxin in the 4th flask was probably due to the fact that on the 7th day a fairly good membrane had formed. After the 9th day a better membrane appeared on the 3d flask and that on the 4th partly subsided. As a result, the 4th flask lost while the 3d gained in toxin as shown by the second test made on the 15th day. The uncertain yield of bacilli without de-

cided membrane-forming capacity as well as the disadvantages of prolonged stay in the thermostat are here unexpectedly demonstrated.

TABLE II.

Culture used.	Peptone in per cent.	Dextrose in per cent	Initial reaction.	Reaction (after 9 days).	Reaction (after 15 days).	Bacillus No. 12. (Toxicity after 9 days, with * after 15 days.)	Estimated m. f. d. for 250-280 gramme guinea-pig.
α	2	.11	−.75	+.5	—	.02 cc. fatal to 411-gramme guinea-pig in 3½ days	.015 cc.
α	1	.11	−.8	+.5	—	.25 cc. nearly fatal to 383-gramme guinea-pig. Large slough	.025
β	2	.11	−.75	−.65	+.1	.05 cc. fatal to 268-gramme guinea-pig in 6½ days	.05
						.03 cc. produces moderate necrosis only	
						*.04 cc. fatal to 278-gramme guinea-pig in 5 days	.04
β	1	.11	−.8	−.7	+.1	.03 cc. " 353 " " 4½ "	.025
						*.04 cc. " 267 " " 2½ "	.036

It must not be inferred from these experiments that the membrane-forming bacillus is capable of overcoming the obstacles inherent in unfermented bouillon. The most favorable conditions under which the bacillus of Park and Williams, grown continuously in bouillon, may multiply in unfermented bouillon do not bring the toxicity up to the concentration obtainable in fermented bouillon to which dextrose has been added. This is clearly shown by the following tests of 5 different lots of unfermented bouillon recently made under the same conditions as those governing the experiment in Table I. The acid reaction was in all cases reduced by adding normal Na_2CO_3. The flasks were inoculated with the culture kept growing in bouillon only. The column headed "Muscle-sugar test" indicates the acidity of the closed branch of the fermentation-tube culture of *B. coli* over and above the acidity of the sterile bouillon. Allowing an increase of

acidity of 1 per cent for 0.1 per cent of sugar* it will be seen that the amount is between 0.09 and .17 per cent—not more than the amount of dextrose that is easily managed and utilized by diphtheria bacilli.

TABLE III.

Designation of bouillon.	Muscle-sugar test.	Initial reaction.	Final reaction after 11 days.	Result.
A	1.3	−.85	+0.	.02 cc. fatal to 244-gramme guinea-pig in $1\frac{3}{4}$ days ± m. f. d. = .012 cc.
B	1.1	−8.5	−2.	.02 cc. fatal to 243-gramme guinea-pig in $1\frac{1}{2}$ days ± m. f. d. = .01 cc.
C	1.4	−.8	−1.5	.02 cc. fatal to 245-gramme guinea-pig in 30 hours. .008 cc. " 253 " " " $2\frac{1}{2}$ days ± m. f. d. = .007 cc.
D	0.9	−.8	−1.8	.01 cc. fatal to 251-gramme guinea-pig in $2\frac{2}{3}$ days. m. f. d. = .009 cc.
E	1.6	−0.7	+.15	.01 cc. produces large slough in 265-gramme guinea-pig. m. f. d. = .012 cc.

Tables I and III show that $\frac{1}{2}$ to $\frac{1}{5}$ of the amount of toxin obtainable in fermented bouillon plus dextrose is produced in ordinary bouillon under identical conditions. For bacilli without the training for surface growth the result would have been far worse, as all experimenters have amply testified.† Table III also shows that the bacillus employed, in spite of its surface growth, was unable to make more than 2 out of 5 lots of bouillon alkaline within 11 days. Nevertheless, a considerable amount of toxin had accumulated. The toxin production goes on in spite of unfavorable conditions, probably in virtue of the persistent membrane growth and the large amount of available peptone. The table furnishes further illustration of the fact that a strongly alkaline end reaction does not necessarily imply the greatest toxic accumulation. A comparison of this with preceding tables

* See *Journ. Boston Soc. Med. Sciences*, June, 1898, for this estimate.

† Dr. W. H. Park informs me that he obtains no better results than these with unfermented bouillon at the present time.

shows that 1 per cent peptone or even less in fermented bouillon may accomplish more than 2 per cent in ordinary bouillon.

The oxygen supply.—Concerning the need of abundant oxygen in cultures of diphtheria bacilli there is general agreement and but little need be said. Ventilation is readily secured in the modified Fernbach flask shown in Fig. 1 (p. 381), and this flask I have invariably found superior to the other forms of the Fernbach and to Erlenmeyer flasks when the layer of culture fluid was about 2.5 cm. deep. By reducing the depth of the layer these flasks become more efficient.

CONCLUSIONS.

1. Dextrose is not in itself injurious but rather favorable to toxin production. When added in quantities not exceeding 0.2 per cent to peptone bouillon freed from fermentable acid-producing substances (muscle sugar) it leads to a maximum accumulation of toxin by utilizing the available peptone to the best advantage.

2. The different courses taken by cultures of diphtheria bacilli in ordinary unfermented peptone bouillon containing muscle sugar and in peptone bouillon made from fermented infusion to which 0.1 to 0.2 per cent dextrose has been added are manifested by an increased production of toxin in the latter as well as by a rapid return from an acid to an alkaline reaction. In the former an acid reaction may prevail even under most favorable conditions.

3. These differences may be explained by assuming either that the acid products of the muscle sugar are different from those of dextrose and non-utilizable, or else that the bouillon contains certain other unknown inhibitory substances removed during fermentation. The use of synthesized media and an analysis of the acid products in fermented bouillon plus dextrose and in unfermented bouillon would aid in explaining the differences.

4. Among the accessory conditions which favor the toxin production in unfermented bouillon, as pointed out by Park and Williams, are increased quantities of peptone, well developed surface growth of the diphtheria bacilli, and a low initial acid reaction (phenolphthalein). In fermented bouillon these accessory conditions are also favoring, though of less importance.

THE ORIGIN OF FAT FROM PROTEIN IN THE SO-CALLED FATTY METAMORPHOSIS OF PHOSPHORUS POISONING.

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The idea of employing hibernating animals for the study of fatty degeneration seems to have originated with Leo (1). Later Polimanti (2) took up the plan along similar but less uncontrolled lines, and reached the same conclusions as Leo,—that in phosphorus poisoning in frogs fat is formed from the protein. In the work of both were obvious errors and also opportunities for further error. Polimanti, as Pflueger (3) promptly pointed out, erred in not having determined the initial weight of his live frogs, and in assuming that the weight at death and the dried residue of the poisoned and control frogs could be properly compared; he erred further in employing heavier frogs for the poisoning, while lighter frogs were used as controls; and most important of all, Pflueger demonstrated that hibernating frogs contain sufficient glycogen to account for the fat apparently formed. In such experiments it must obviously be demonstrated that the fat in the tissues of a poisoned animal could not be derived from any other source than protein, before it can be held as demonstrated that in fatty degenerations fats are produced from protein.

I have repeated the experiments of producing phosphorus poisoning in frogs, and, I believe, have arranged the conditions so rigidly that the results must be indubitable, in so far at least as this particular procedure is concerned. I have utilized the Rana palustris.

The frogs were gathered about the first of December, 1898, and held in moist chambers exposed to the light for nearly four months at a temperature of 18-20° C. This was done in order to exhaust as much as

possible the store-houses of fat and glycogen. Twenty-eight males were then selected, and divided into groups of fourteen each; the total weight of each group was almost identical with that of the other, and the frogs were so paired as to make the series balance well. The frogs were wiped dry with filter paper, the urine expressed, and they were then carefully weighed. The frogs of one group were then poisoned by phosphorus. I wished to prolong the action of the phosphorus, and the frogs were given two small doses rather than one large dose. The phosphorus was administered in an emulsion of acacia, and each frog was given 0.001 grm. at each administration, the second dose being given on the fourth day. Whenever a frog died his control fellow was at once killed. Such as did not die were killed upon the twelfth day, and their controls at the same time. The animals were weighed when found dead or when killed. An interesting point was the œdematous condition of many of the poisoned frogs, especially those found dead over night. The original weights were as follows:

CONTROLS.	TO BE POISONED.
C1=21.990	P1=22.450
C2=20.600	P2=21.000
C3=21.330	P3=21.000
C4=22.850	P4=21.690
C5=20.090	P5=20.620
C6=21.200	P6=21.450
C7=38.000	P7=43.700
C8=28.030	P8=26.770
C9=19.870	P9=21.100
C10=19.850 +	P10=18.280
C11=18.150	P11=16.260
C12=14.950	P12=15.480
C13=14.320	P13=14.150
C14=12.840	P14=10.400
294.430 grm.	294.350 grm.

One control frog, C10, weight 19.850, was lost. The total weight of the dead animals was:

CONTROLS.	POISONED.
C1=19.300	P1=22.100 killed.
C2=19.850	P2=22.720 dead.
C3=20.270	P3=25.480 dead.
C4=22.500	P4=21.200 killed.
C5=20.250	P5=24.100 dead.
C6=21.000	P6=21.450 killed.
C7=36.150	P7=42.500 killed.
C8=25.650	P8=27.900 dead.
C9=16.650	P9=20.900 killed.
C10=lost	P10=18.900 killed.
C11=17.300	P11=13.900 dead.
C12=12.900	P12=14.450 killed.
C13=14.400	P13=13.500 killed.
C14=12.500	P14=13.000 dead.
258.720	302.100 grm.
19.000 lost, approx.	
277.720 grm.	

In all probability much of the loss in weight in the control frogs was due to the fact that the dead frogs could be wiped more completely dry than the living frogs. Poisoned frogs Nos. 2, 3, 5, 8, and 14 gained notably in weight; they were œdematous and the gain seemed obviously water. A comparison of these tables of weights illustrates how inaccurate it would be to use as the basis of calculation the dead weight of the frogs at the end of the experiment. The only correct basis of calculation is the original live weight before the experiment.

The bodies of the frogs were first partly dried in ovens at 70° C., and then completely dried in a vacuum over sulphuric acid. They were then finely ground in a small closed hand-mill, again dried in a vacuum over sulphuric acid, and weighed. The dried residues were:

Control frogs	Poisoned Frogs
Control frogs: 49.855 grm. or 18.15% of the original weight.	Poisoned Frogs: 44.620 grm. or 15.15% of the original weight.

In order to make the comparison complete, allowance must be made for the lost control frog: assuming his dried residue the same as his fellows the dried residue of the control frogs was 18.15 per cent of 294.130, or 53.441 grm., as against 44.620 grm. for the poisoned frogs, representing a loss of dried residue during the process of poisoning of 8.821 grm., or 16.5 per cent.

Three portions each of the control and poisoned frogs were then submitted to the Kjeldahl process for the estimation of nitrogen. The results were as follows:

CONTROLS:	POISONED:
1—0.604 grm. subs. = 0.0708 N.	1—0.735 grm. subs. = 0.0865 N.
2—0.570 grm. subs. = 0.0666 N.	2—0.550 grm. subs. = 0.0645 N.
3—0.575 grm. subs. = 0.0656 N.	3—0.650 grm. subs. = 0.0766 N.

On an average, 11.55% of the dried residue, or 5.762 grm. total nitrogen. Including the lost control frog, however, the dried residue was 53.441 grm., of which the total nitrogen (11.55 × 53.441) would amount to 6.403 grm., or 2.173% of the original weight. This with the factor 6.25 would correspond to a total protein of 40.019 grm.

An average of 11.70% of the dried residue, or 5.221 grm. total nitrogen, or 1.776% of the original weight. This with the factor 6.25 would correspond to a protein of 32.631 grm.

Thus during the process of the poisoning by phosphorus 1.182 grm. of nitrogen were lost as compared to the control frogs; this corresponds to a loss in protein, using the factor 6.25, of 7.388 grm., or 18.37 per cent. These 7.388 grm. of proteid contain about 3.500 grm. of carbon, which could conceivably correspond to about 4.600 grm. of fat.

The material of each series was then subjected to the same analyses, as follows:

The entire remainder of the dried residue, after the small portions for the nitrogen determinations had been taken, was placed in a beaker, 500 cc. of water added, hydrochloric acid until the mixture contained one-half of one per cent HCl, and 2 grm. of pepsin which was free of fat and glycogen. The beaker was then placed in an oven at 38° C. and allowed to remain there with the further addition of HCl in the third day, until digestion was completed, which was at the end of the fourth day, when the frog powder had gone into solution except for a few minute particles. The acidity was then partially neutralized by the addition of NaHO, and the fluid transferred into modified Sohxlet tubes designed for the extraction of fats from liquids, and there extracted with ether for about seven hours per day during five days. To make sure of complete extractions the fluid was then thoroughly shaken with ether in glass-stoppered cylinders. The combined ethereal extractions were then allowed to evaporate, and the fat collected in pure ether, filtered, the ether allowed to evaporate, the fat then placed in an oven at 95° for several days, cooled and weighed as crude fat. The crude fats were then saponified with alcoholic KHO, the mixture rendered acid with H_2SO_4, and the fatty acids extracted with ether. The ether was then allowed to evaporate, the fatty acids then dissolved in alcohol, and titrated with N/10 alcoholic potassium hydrate, with phenolphthalein as an indicator. From the titration the fats were calculated upon the basis of a mixture of stearin, palmatin, and olein, a calculation certainly not accurately applicable to frog's fat. Of the fat in the control frogs, 4.534 grm., 86.1 per cent was fat, being 3.904 grm.; the remainder, 0.630 grm., being lecithin, cholesterin, pigments, etc. Of the fat in the poisoned frogs, 3.508 grm., 84.6 per cent was pure, being 2.968 grm.; the remainder, 0.540 grm. being lecithin, cholesterin, pigments, etc. Thus of pure fat the control frogs had 3.904 grm., the poisoned frogs, 2.965 grm., a loss of 0.936 grm. The chief value of this study of the fats lies in the fact that it demonstrated that the ethereal extracts from the two series contained approximately the same amount of fat.

The weighed fats were not deeply pigmented. The fat from the control frogs weighed 4.0846 grm. The fat from the poisoned frogs weighed 3.356 grm. Since these quantities were obtained from the powders minus the quantities used for the nitrogen analysis, they must be corrected. From the residue of the control frogs, 49.885 grm., 1.749 grm. were removed from the three nitrogen analyses; the 4.0846 grm. fat in the control frogs were therefore 48.136/49.885 of the total, which would

be 4.232 grm. From the dried residue of the poisoned frogs, 44.620 grm., 1.935 grm. were removed for the nitrogen analyses; the 3.356 grm. fat were therefore 42.685/44.620 of the total, which would be 3.508 grm. The 4.232 grm. of fat in the control frogs is, however, still too small by reason of the absence from the analysis of the one lost frog. As calculated for the dried residue, this was raised from 49.885 to 53.441 grm.; the 4.232 grm. of fat therefore represented 49.885/53.441 of the real total, which would be 4.534 grm. Since 294.430 grm. of control frogs contained 4.534 grm. of fat, the percentage was 1.54 per cent; and since 294.350 grm. of poisoned frogs contained 3.508 grm. of fat, the percentage was 1.19 per cent. Thus the control frogs contained 4.534 grm. of fat, and the poisoned animals 3.508 grm.—a loss of fat, therefore, during the course of the phosphorus poisoning, of 1.026 grm.—or 22.64 per cent.

Following the extraction of the fats the digested fluid was neutralized, wherein a rather heavy precipitate was produced. The precipitate was separated by filtration, and in the filtrate and precipitate the Brücke-Külz method for glycogen was conducted separately. The use of this method after the digestion of the substance has been recommended by Austin (4).

The precipitate was heated in a 2 per cent KHO until homogeneous, then cooled and HCl and Brücke's reagent added until no further precipitate appeared. The precipitate was then filtered off, redissolved in 2 per cent KHO, reprecipitated with HCl and Brücke's reagent, and this process was repeated four times; the filtrates were then united.

The original filtrate was then mixed with twice its volume of 96 vol. per cent alcohol and allowed to stand 24 hours. By this time the glycogen was entirely precipitated, and it was hoped that the albumoses would remain entirely in solution. This they did not do; a portion was precipitated. The precipitate was washed twice in 62 vol. per cent alcohol containing a little NaCl, and then dissolved in warm water. In order to attempt to avoid the milky cloudiness which would surely be produced by the Brücke reagent in a liquid containing albumoses, the solution was again mixed with double its volume of 96 vol. per cent alcohol; as before, albumoses were precipitated and could not be separated from the glycogen in this way. Upon the following day, the precipitate was dissolved in warm water, the required amount of KHO added to bring it up to 2 per cent KHO, and then submitted to the precipitation with HCl and Brücke's reagent; a small precipitate and a milky cloudiness appeared, and the solution was allowed to stand 24 hours, but the cloudiness still persisted. Thereupon the entire Brücke-Külz procedure was repeated, but with the same result. This time the precipitate was sepa-

rated by filtration, and four times redissolved in KHO, reprecipitated by HCl and Brücke's reagent and filtered. All the filtrates were united and finally added to the filtrates obtained from the manipulation of the original residue, and thus all the glycogen in each series of frogs was finally brought into one solution. This solution was of a pale milky color, and all efforts to clarify it were fruitless. The same phenomena occurred in both series of analyses, and were in all probability due to albumoses which arose during the digestion of the frogs. It was obvious that the glycogen would not be pure preparations. Double analyses of aliquot parts of each solution were then made according to the Külz (5) method—precipitation by 2 volumes of 96 vol. per cent alcohol, careful washing upon the weighed filter paper, first with 66 per cent alcohol, then 95 per cent, three times with absolute alcohol, three times with ether, and finally again with absolute alcohol, and drying to a constant weight at 90° C., which required about four days. The glycogen in the control frogs was 2.029 grm. and 2.070 grm. respectively for two analyses, the average being 2.049 grm. The glycogen in the poisoned frogs was 1.784 grm. and 1.802 grm. respectively for two analyses, the average being 1.793 grm. These must both be corrected, as were the fats, for the quantity of dried residue removed for the nitrogen analyses. The 2.049 grm. in the control frogs was 48.136/49.885 of the total, therefore 2.123 grm. The 1.793 grm. in the poisoned frogs was 42.685/44.620 of the total, therefore 1.874 grm. The 2.123 grm. in the control frogs must be further corrected for the lost frog; it was 49.885/53.441 of the real total, which was therefore 2.274 grm.

It was obviously necessary to determine the purity of the glycogen. This was done by inverting it and making a quantitative analysis of the sugar. For inversion I employed a 2.5 per cent HCl solution, using a boiling water bath for four hours. The sugar was determined by the cupric oxide method as elaborated by Pflueger (6), a method I have repeatedly employed with entire satisfaction. According to the formula for glycogen of Hueppert (7), 11 parts of glycogen should furnish 12 parts of dextrose. Of the glycogen from the control frogs I inverted 0.109 grm., which should have produced 0.119 grm. dextrose; my analysis furnished 0.102 grm.; the glycogen was therefore 85.8 per cent pure; 85.8 per cent of the glycogen in the control frogs, 2.274 grm., equals 1.951 grm., the final figure for glycogen in the control frogs, 0.66 per cent of the original weight of the frogs. Of the glycogen from the poisoned frogs, 0.0942 grm. were inverted, and should have produced 0.102 grm. of dextrose; the analysis furnished 0.0924 grm., the glycogen from the poisoned frogs was therefore 91.5 per cent pure; 91.5 per cent of the

glycogen in the poisoned frogs, 1.817 grm., would amount to 1.690 grm., which is the final figure for the glycogen in the poisoned frogs, or 0.57 per cent of the original weight of the frogs. Thus during the course of the phosphorus poisoning the frogs lost 0.261 grm. of glycogen, or 13.3 per cent. As a matter of fact, since all the glycogen results are too low—the Külz method giving always too low results—the loss in glycogen was probably greater.

SUMMARY AND DISCUSSION.

588.780 grm. of frogs, all of the same sex, of the same comparative approximate weights, taken from the ground about the same time, kept awake and without food for nearly the same time, were divided into equal groups; the one group was poisoned with phosphorus, the other group held as a control. The frogs in the poisoned group *lost* in dried residue 8.821 grm. or 16.5 per cent of the dried residue of the control group; 1.182 grm. of nitrogen, corresponding to 7.388 of proteid, or 18.45 per cent of the nitrogen and protein in the control frogs; 1.026 grm. of fat, or 22.64 per cent of the fat in the control animals; and 0.261 grm. glycogen, or 13.3 + per cent of the glycogen in the control frogs.

I believe that it is obvious that in these experiments no fats were produced from protein. Mathematically, it is possible to conceive that fats could have been formed but entirely burned up. As previously stated, the carbon in the proteid lost during the poisoning was equivalent to 4.600 grm. of fat, and it is conceivable that these 4.600 grm. of fat were formed, but that they, together with the 1.026 grm. of fat actually lost during the experiments, were burned. In brief, the fat combustion might have been tremendously increased, and masked an actual fat formation. This however is unsupported by evidence, and is highly improbable. It is hard to conceive that in an organism whose katabolic functions were greatly augmented as the result of phosphorus poisoning, in which protein, fat, and glycogen were being burned in excess, the carbon of the protein would first have been converted into fat and then the fat burned as such. I believe the only conclusion which can be drawn from these experiments is that no fat was formed as the result of phosphorus poisoning. Thus the fatty

degenerations so-called which occurred in these frogs did not comprehend any formation of fat at all, but simply the deposition of fat.

These results are directly opposite to those of Polimanti. Polimanti apparently did not weigh his animals before the beginning of the experiment, and based his calculations upon the relation of the fat to the dried residue. Obviously his calculation was based upon the assumption that the dried residue of a frog was unaffected by phosphorus poisoning. Polimanti, in declining to base his calculations upon the weight of the animals when dead, states that as water is often increased, such a calculation would be misleading. But since the dried residue may and does vary, calculations based upon it are also misleading, and thus the only proper basis of calculation is the original weight of the frogs before the experimentation. Calculated upon the basis of the dried residue, in my material the percentage of fat in the control animals was 8.48 per cent, in the poisoned animals 7.86 per cent, so that, even upon the basis of Polimanti's incorrect calculation, in my experiments fat was lost in notable quantity.

Just before this study was completed, the publication of Athanasiu, (8) from Pflueger's laboratory, appeared. Operating with a large number of frogs, and under varying conditions, with careful methods and rigid controls, Athanasiu reached the conclusions: that phosphorus poisoning has no effect upon the total quantity of fat in frogs; that it has little effect upon the nitrogen; that it produces a diminution in the quantity of glycogen; and that the fatty degenerations are really fatty infiltrations. While my results agree with those of Athanasiu in the essential point that no fat was produced by phosphorus poisoning, they differ in that the poisoned frogs, in my experiments, lost fat and protein as well as glycogen, while his frogs lost only glycogen. Since our methods were almost the same, the differences must have resided either in the conditions surrounding the experiments, or in the animals. I do not believe that such differences exist between the Rana fusca and esculenta of Europe and the Rana palustris of America as to explain the differences in our results. These differences I believe may be explained by varying conditions. My animals were kept in a warm cellar, at a temperature

of from 18 to 20° C. The period of poisoning with Athanasiu's frogs varied from one to six days; all of my frogs lived over six days, most of them ten or twelve days. Since we know that the katabolic actions of most poisons are greater in prolonged intoxications, it is fair to assume that the time element was the factor in the production of my results.

While it would be unscientific and illogical to state that fat cannot be formed from protein, the fact stands that it has never been shown, either in physiology or pathology, that fats are formed from protein. On the contrary, nearly all of the careful work upon the question has yielded negative results. Not only has it never been shown that, in fatty degeneration so-called, fat is formed from the cellular protein, but it has never been demonstrated that fat is then formed at all, even from glucosides, etc., substances from which fats may be readily formed.

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2. Polimanti.—Pflueger's *Archiv*, 1898, lxx, 349.
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A CONTRIBUTION TO THE KNOWLEDGE OF THE PATHOLOGY OF FRAGMENTATION AND SEGMENTATION, AND FIBROSIS OF THE MYOCARDIUM.

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PLATES XXI AND XXII.

In order to make clear the following description of the minute changes taking place in the heart-muscle cell, it will be well first to describe briefly the appearances met with in normal cardiac muscle. In a previous paper* I have given a detailed description of these, together with an account of the histogenesis of the cardiac muscle cell.

HISTOLOGY OF THE MUSCLE OF THE NORMAL HEART.

The normal adult heart muscle of the human subject is made up of irregular rhomboidal cells, which are usually considerably branched. Each cell consists of darkly staining columns, which run longitudinally and are separated by unstained substance. These columns are commonly spoken of as fibril bundles, and correspond with what v. Kölliker has called "Muskelsäulchen." The unstained substance between is generally known as sarcoplasm.

Careful observation and certain methods of special staining reveal a definite relation between these two parts of the cell. The fibril bundles are striated like voluntary muscle, showing a narrow disc called Krause's membrane, and a broader disc or Brücke's line of doubly refractive substance between each two narrow striations. Krause's membrane corresponds with the "Zwischenscheibe" of German writers, and Brücke's line is identical with the "Querscheibe."

* J. B. MacCallum, On the Histology and Histogenesis of the Heart-muscle Cell, *Anatomischer Anzeiger*, 1897, xiii, 609.

In thin sections, especially those stained by Kolossow's method, the Krause's membranes are seen to belong to the sarcoplasm as well as to the fibril bundles (Plate XXI, Fig. 1). The sarcoplasm is divided into distinct discs by membranes which horizontally are continuous with the Krause's membranes of the fibril bundles. There may be more than one of these discs between two adjacent fibril bundles (Plate XXI, Fig. 1, A); and at the centre of the cell the perinuclear sarcoplasm is made up of discs alone.

Seen in transverse section (Plate XXI, Fig. 2) the cell consists of darkly staining masses which are cross-sections of fibril bundles, separated by definite discs of unstained substance, the sarcoplasmic discs. The muscle fibre then contains a continuous network, made up of the fibril bundles, and the membranes bounding the sarcoplasmic discs. The points of junction of these membranes with the fibril bundles are at the narrow striations or Krause's membranes of the fibril bundles.

The relation of these parts is made clear by their histogenesis. The earliest stage in the development of the heart-muscle cell shows an irregular network in the protoplasm. This tends to become more regular as shown in Plate XXI, Fig. 3, assuming the form of discs which in transverse sections are seen as clear circular areas. Some of these discs break up into smaller ones, and in the angles between them there is an accumulation or a differentiation of the substance of the network, giving rise to longitudinally disposed masses. The earliest formation of these is around the periphery of the cell as shown in Plate XXI, Fig. 4. They become what in the adult cell are known as fibril bundles, and the discs left in between are the sarcoplasmic discs described. It is thus seen that the network, composed partly of the fibril bundles, is derived directly from the primitive network of the embryonic cell. It is further a very noticeable fact that the formation of fibril bundles takes place first at the peripheral part of the cell, so that those fibril bundles which are formed latest are nearest the centrally placed nucleus.

In the state of extension the muscle fibre differs very markedly from the condition described above. It often happens, especially in cases

of fragmentation of the myocardium, that two cells, separated only by a cement line, are in the state of contraction and extension respectively. The contracted fibre is much broader than the other, and, on following it to the cement line, the edges suddenly narrow down when the next cell is reached, as shown in Plate XXI, Fig. 5. It is evident that the narrow cell is the extended one, and the histological characters of it indicate that it has undergone a very decided change. The most noticeable differences are the following:—In the extended fibre the lateral edges are distinctly concave, as compared with the slightly convex boundaries of the contracted cell. The fibril-bundles are much closer together in the extended fibre, and seem to occupy relatively more space than in the contracted condition. As a consequence of this the sarcoplasm is much less in evidence. Within themselves also the fibril bundles show marked changes. The distance between each two narrow striations or Krause's membranes is very much greater than in contracted muscle. In this space there is a darkly staining mass which has on each side, between it and the respective Krause's membranes, a clear space. This dark body corresponds in position with the secondary striation, or Brücke's line. It is, however, very different in form from this structure in contracted muscle. It is generally somewhat narrower, and considerably longer. Whereas in contracted fibres its transverse diameter is two or three times as great as its length,in extended muscle it is either equally great in both axes, or even greater in the long axis. As represented in Plate XXI, Fig. 5, B, the most conspicuous striation in contracted muscle is Krause's membrane; while in the extended condition (Fig. 5, A) Brücke's line becomes very distinct, and the narrow striations are hardly to be seen.

METHODS AND MATERIAL.

For the following study of some of the pathological conditions met with in heart muscle, only human tissues were used. These were obtained in part fresh from autopsies made in the Johns Hopkins Hospital, and in part from already preserved specimens. With regard to some of the tissues used in the study of fragmentation and

segmentation, I am greatly indebted to Dr. Hektoen of Chicago, who, at the request of Dr. Flexner, sent a number of exquisite specimens to the laboratory.

All the tissues that were obtained fresh were treated by Kolossow's osmic acid method,* and also by the ordinary methods. The other tissues were hardened in Zenker's fluid, alcohol or Müller's fluid. Sections were cut in both celloidin and paraffin.

The tissues treated by Kolossow's method were studied without further staining. The others after being cut were submitted to a treatment somewhat similar to that described in Kolossow's method. The sections were placed in 1 per cent osmic acid for 2—5 minutes until they were thoroughly impregnated. They were then transferred to Kolossow's reducing fluid (tannic and pyrogallic acids) until the precipitation was complete. If the stain is not intense enough, this process may be repeated, care being taken, however, that all the reducing fluid is washed out with water before the sections are again put into osmic acid. The sections may be dehydrated and mounted in the ordinary way. This mode of staining is not by any means recommended in place of Kolossow's original method, for it gives much less distinct pictures. It is, however, convenient, in that tissues hardened by any of the ordinary methods can be used, while in the original method only fresh tissues can be employed. A nuclear stain may be obtained in connection with this, by treating the sections subsequently with safranin.

Specimens stained in hæmatoxylin and eosin, safranin, and van Gieson's fluid were also studied.

FRAGMENTATION AND SEGMENTATION OF THE MYOCARDIUM.

Renaut and Landouzy† in 1877 were the first to describe a dissociation of the muscle cells of the heart. They believed that it was due to nutritive disturbances, which acted in such a way that the cement substance between the cells was softened. They called the condition "segmentation."

*A. Kolossow, Ueber eine neue Methode der Bearbeitung der Gewebe mit Osmiumsäure, *Zeitschr. f. wissenschaftl. Mikroskopie*, 1892, ix, 38.

† Renaut, Note sur les altérations du myocarde accompagnant l'inertie cardiaque, *Compt. rend. Société de Biologie*, July, 1877.

Renaut* in 1890 contributed another article in which he considers the condition as a definite disease; and attributes the separation of the cells to a solution of the cement substance.

Von Recklinghausen † believes that breaking may take place in the cell-body as well as in the cement line, and that it is due to irregularities in contraction, rather than to a solution of the cement substance.

Israel‡ observed that there is a separation of the muscle bundles, suggesting that the change is brought about by mechanical influences.

Browicz§ believes that the dissociation of the muscle cells occurs before death, and may give rise to insufficiency or even stoppage of the heart.

According to Oestreich|| breaking takes place mainly in the body of the cell, although it may occur in the cement line. The condition cannot be brought about by decomposition, and all the evidence points to its being an ante-mortem process. Oestreich cites instances of its presence in sudden deaths, death under chloroform, etc. It may occur in almost any disease.

Bard¶ believes that the breaking occurs after death, in muscle which is abnormally fragile.

It is thus believed by some, particularly Browicz, Renaut, Tedeschi, Israel, and Durand** that there is some chemical substance formed which acts on the cement leading to its solution.

By others, especially v. Recklinghausen, Oestreich, and Bard, the condition is thought to be brought about mechanically.

Since these works a paper has appeared by L. Hektoen,†† in which the subject is carefully worked over. Hektoen divides the process definitely into segmentation and fragmentation. The former term is used when breaking on the cement line is meant, while "fragmentation" indicates

* Renaut, *Gaz. méd. de Paris*, 1890, 7. s., vii, 109; 123.

† Von Recklinghausen, *Verhandlungen des X Intern. Congress., Berlin* (1890), ii, Abth. 3, p. 67.

‡ Israel, Zur Entstehung der Fragmentatio myocardii, Virchow's *Archiv*, 1893, cxxxiii, 551.

§ Browicz, Ueber die Bedeutung der Veränderungen der Kittsubstanz der Muskelzellbalken des Herzmuskels, Virchow's *Archiv*, 1893, cxxxiv, 1.

|| Oestreich, Die Fragmentatio myocardii, Virchow's *Archiv*, 1894, cxxxv, 79.

¶ Bard, De la signification anatomique et clinique des diverses lésions du myocardè, *Congrès Français de Médecine*, Paris, 1895, p. 806.

** Durand, Étude anatomique sur le segment cellulaire contractile et le tissue connectif du muscle cardiaque. Thèse, Lyon, 1879.

†† L. Hektoen, Segmentation and Fragmentation of the Myocardium, *American Journal of Medical Sciences*, 1897, cxiv, 555.

breaking on the body of the cell. The two conditions, he thinks, are due to a "disproportion between the vigor and order of muscular contraction and muscular cohesion." According to him fragmentation is possibly, if not probably, due to mechanical agencies; while segmentation cannot be produced in this way.

In a recent paper, Karcher* has reported more especially on experimental fragmentation. He notices the increase in pigment, the swelling of the nuclei, and the changes in the cement substance. His findings confirm the earlier views that the condition is most commonly found in the papillary muscles, less frequently in the left ventricle, and only occasionally in the right ventricle. According to Karcher the causes may be separated into two classes:

1. Fragmentation as the result of sudden injuries or acute processes.
2. Fragmentation following chronic diseases when there is a disturbance of the nutrition of the heart.

Experimentally Karcher obtained fragmentation by cutting the cervical cord and stimulating the peripheral cut end; also by cutting the vagus in the cervical region, and subsequently giving strychnine. He concludes that fragmentation of the myocardium is caused chiefly by disturbances in the nutrition of the heart, especially when associated with a sudden rise in blood pressure.

Histology of the heart muscle in fragmentation and segmentation of the myocardium.

Studied with high powers and with the special methods of staining described, the condition known as fragmentatio myocardii is seen to be a more complex process than a mere breaking of the fibre. In every case where the breaking is at all prominent, there is a peculiar condition of the muscle which consists in an alternating contraction and extension of the fibres. Extension and contraction are not here, as is the case in normal muscle, present in large areas. The cells seem to be affected separately, and one often finds a contracted fibre immediately adjacent to an extended one. In normal muscle the two conditions may be present in the same section, but in such a case all the fibres in one part of the section are contracted and all those in another part are extended. The muscle of fragmentation, however, shows a

* J. Karcher, Ueber die Fragmentation des Herzmuskels. *Deutsches Archiv für klinische Medicin*, 1897, lx, 67.

remarkable distribution of the differently affected fibres. It is very often possible to follow a contracted fibre down to the cement line, and find the cell on the other side of the line suddenly narrowed down to the extended condition, as described above. In such a place the contracted fibre would be separated only by a cement line from the extended one. The same thing is seen even in different parts of the same cell. Bands of contracted and extended tissue alternate with one another, so that in extreme cases the fibre has a mottled appearance. This appearance is certainly pathological, and it is, to say the least, strange to see in the protoplasm of the same cell, two such distinct histological conditions as extension and contraction of muscle fibres.

The breaking which accompanies this condition can be classed under four headings, according to its position and histological character:

1. Breaks occurring in the cement line.
2. Breaks occurring in contracted muscle.
3. Breaks occurring in extended muscle.
4. Breaks occurring as the result of a degenerative process, whose initial stage is an extreme extension of the muscle.

We may then speak of simple fragmentation and degenerative fragmentation.

Histology of simple fragmentation.

This condition is known as segmentation when present in the cement lines, and as fragmentation when the breaks occur in the body of the cell. In segmentation the break takes place in the centre of the line, leaving on either side the "stratum granulosum terminale," to use a term introduced by Przewoski,* which limits the end of the fibre. In the body of the cell the fragmentation differs somewhat according as the muscle is contracted or extended.

(*a*) *Simple fragmentation in contracted muscle.*—The break is irregular and tends to take a stair-like direction (Plate XXI, Fig. 6, A). It is usually a clear break which passes across the breadth of the fibre. It is difficult to determine exactly where the fracture occurs in rela-

* Przewoski, Du mode de réunion des cellules myocardiaques de l'homme adulte, *Archives des sciences biologiques de St. Pétersbourg*, 1893, ii. p. 286.

tion to the fibril bundles and sarcoplasmic discs. It is certain, however, that it is very close to, if not actually in, Krause's membrane. In nearly every instance one can make out this structure at the end of the broken fibril bundle, so placed that it limits the bundle and sarcoplasm at the line of fracture. The line of Brücke is never seen at the broken end of the fibril bundle, and the tendency seems to be for the break to occur always at Krause's membrane. Further, it is always at right angles to the long axis of the fibril bundles, that is to say in the line of Krause's membrane.

(*b*) *Simple fragmentation in extended muscle.*—This condition is much more extensive than the fragmentation just described. There seems to be less resistance against the forces that cause breaking than in contracted muscle. One often sees small breaks on either side of the main one, and there may even be separated fragments of fibril bundles in the line of fracture. As shown in Plate XXI, Fig. 6, B, there is usually an area made up of segments partially or entirely broken off from the fibril bundles. Here also the breaking seems to take place at Krause's membrane, for all the fragments are bounded by this line, as represented in the figure referred to.

Histology of the sarcolytic degeneration of fragmentation.

Thin sections, stained by the methods mentioned above, but particularly by Kolossow's osmic acid method, show definite areas of the muscle fibre from which some of the structural elements seem to have disappeared. These areas stain faintly as compared with the rest of the cell, and present a more or less marked dissociation of the protoplasmic structure. Every gradation can be made out between still solid tissue and areas which are merely a mass of detritus. This condition is shown in Plate XXI, Fig. 7, and will be spoken of as sarcolytic degeneration. When examined more carefully it is seen that those areas which are least changed show a great resemblance to normal extended muscle (Plate XXI, Fig. 8, A). The fibre, or the part affected, becomes somewhat more narrow than the normal tissue, and the minute characters are the same as those mentioned in the description of normal muscle. The first stage in the process, then, is

one which causes the muscle to undergo a change similar to that undergone by normal muscle extension. A gradual transition can be traced from the primary stage to that in which a complete disintegration of the part takes place. In some fibres the rows of Brücke's lines which run across the whole cell, forming the broad striations, are more distant from one another than in extended fibres (Plate XXI, Fig. 8, D). This appearance suggests that a stretching out has taken place which is not due to the simple extension of the cells. In other cells the rows of broad striations become irregular, often drawn down at one side, so that they run obliquely instead of transversely, as shown in Plate XXI, Fig. 8, D. The sarcoplasmic discs are correspondingly irregular and appear only as refractive oval unstained bodies, between the darkly stained masses, as may be seen in some parts of Plate XXI, Fig. 7. In still other fibres the lines of Brücke are so irregularly placed that they cannot be said to form lines at all. They are scattered without order over the degenerating area, and are separated by the sarcoplasmic discs, which now have no definite relation to the remains of the fibril bundles. Some of the discs have broken down, leaving only a granular material. In these areas the cell has become considerably narrowed, and has the appearance of having been very much stretched. There are other places in the fibres where the remains of the fibril bundles can, with difficulty, be made out (Plate XXI, Fig. 9, A). Such areas show simply an irregular mass which stains faintly, and is made up of the granular remains of broken down sarcoplasmic discs, along with an occasional darkly staining fragment from the disintegrated fibril bundles. These places tend to become narrower in the centre and show very irregular edges. It is probable that such a fibre would offer very little resistance to stretching, and what appears to be the last stage in the process of degeneration is often seen in cells which are completely separated by the breaking away or absorption of this narrow central part (Plate XXI, Fig. 9, B).

There thus seems to be a definite process of degeneration, which begins with an extension of the fibre, and ends in its disintegration and separation into fractions. It seems certain that this is a definite

pathological process, presenting several stages in its course. These several stages, although running imperceptibly into one another, may be grouped in the following way:

1. Simple extension of the fibre or part of the fibre, with a lengthening and narrowing of the part.

2. Stretching of the fibre, with the production of irregularities in the rows of striations on the fibril bundles, and changes in the relations of the sarcoplasmic discs to the fibril bundles.

3. A condition of still greater irregularity in the distribution of the fragments of fibril bundles, accompanied by a disappearance of some of them.

4. A disappearance of all the remains of the fibril bundles, leaving only a mass of partly broken-down sarcoplasmic discs.

5. Complete disintegration with a breaking across of the area.

The exact relation between this process and the simple fragmentation in the contracted and extended muscle, and segmentation, is not clear. The various conditions occur in the same muscle, and indeed are nearly always seen in the same section, so that they are obviously closely connected. The simple breaks, especially those in contracted muscle, do not differ materially from fractures which are sometimes caused artificially, for example in the process of sectioning. Histologically they resemble mechanical breaks. In the extended fibre, fragmentation seems to be a more serious condition; for, as described above, the extension occurs in an abnormal way. In normal muscle, one does not find the curious alternation of extended and contracted fibres which is characteristic of this condition. Breaking is more common in extended fibres than in contracted ones, and this would in all probability be the case if both were caused by mechanical forces.

In degenerative fragmentation, on the other hand, the disintegration is apparently a more or less gradual process. When the various states are present together, one does not hesitate to say that the degenerative process is the main lesion. It is a much more extensive change than the others. In a great many of the specimens examined, it was found in every cell in the section, and sometimes in two or more places in the same cell, as shown in Plate XXI, Figs. 7 and 8. It was found

in practically all the cases of fragmentation which were examined, and in the great majority of cases was the most conspicuous lesion, when looked for with high powers. The simple breaks, although sometimes very numerous, seem to be an accessory lesion. With such an extensive process of degeneration as that described, it is certain that much of the muscle would be incapable of carrying out its functions. In such an event, a great deal of extra work and strain would be thrown on the remaining healthy muscle. It is conceivable that this unusual strain might cause such simple breaks as those described. A mechanical explanation like this is supported by the fact that in some cases of fibrous myocarditis, where there is a great deal of muscle thrown out of function, there is seen a simple breaking which resembles in every particular that described. If this be true, the main lesion in fragmentation of the myocardium is a degenerative process, the sarcolytic degeneration described, while the simple breaks in the various locations are mechanical results of the unusual strain thrown on the muscle which remains undegenerated.

If the entire condition of fragmentation is due to unfavorable changes in the nutrition of the cells, it is difficult to imagine that simple breaks could be caused in this way. The alternating contracted and extended fibres might, however, arise from such a condition, although the exact manner in which this could occur is far from clear. Whether the changed nutrition renders certain parts of the fibre incapable of responding to a stimulus; or whether it acts as a stimulus to other parts, leaving the areas between in an extended condition, can only be the subject of hypothesis. The degenerative process, however, must be due to some definite nutritive change, and, if this be so, the most plausible explanation of the simple breaks is a mechanical one.

MUSCLE-CELL DEGENERATION FOUND IN FIBROUS MYOCARDITIS.

Huchard * describes the muscle-cell in fibrous myocarditis as undergoing atrophy, and vesicular or vacuolar transformation. Both conditions are found at the periphery of the islands of muscle which

* H. Huchard, Étude clinique de la cardio-sclérose, *Revue de Médecine*, 1892, xii, 421 et seq.

are formed by the connective tissue growth. In vacuolar transformation the muscle-cells appear empty in the centre so that in transverse section they have a ring-like appearance. In a later stage the cell dissociates. According to Huchard, this vacuolar degeneration is nothing more than an œdema of the cardiac fibre.

According to Bard and Philippe,* the interstitial growth of connective tissue is mainly around the vessels. The muscle fibres undergo a "fragmentary degeneration" and there is an increase in the pigment.

There are generally recognized in fibrous myocarditis two processes, the disappearance of muscle-cells, and the overgrowth of newly formed connective tissue. The latter process, which is, as pointed out by Weigert, probably a secondary one compensating for the loss of the more highly differentiated muscle-cells, consists in a proliferation of connective tissue from the already formed tissue cells. Its main growth seems to be from the connective tissue of the blood-vessels. These areas appear as localized patches, or strands of grayish tissue scattered over the muscle, and may be the seat of calcification.

In a cross section of a fibrous patch one sees islands of muscle fibres, separated by the ingrowing connective tissue. At the periphery of these islands, there are always to be seen cells which appear with the low power to be empty in the centre, as Huchard has said. With a higher magnification, however, these cells show all stages of a very definite process of degeneration.

Appearances met with in cross section.—When compared with the normal tissue, there are some cells in which the central undifferentiated sarcoplasm is somewhat increased. This sarcoplasm, like that in normal cells, consists of small discs. In other cells, a large mass of these discs is present in the centre, surrounded by a very much diminished number of fibril bundles. This mass is usually quite irregular in form but its position is, roughly speaking, in the centre of the cell. One also finds cells where there is only a single row of fibril bundles around the periphery while the rest of the cell is made up entirely of sarcoplasmic discs (Plate XXII, Fig. 10, A). Another appearance often met with is shown in Plate XXII, Fig. 11, where the

* L. Bard and Cl. Philippe, De la myocardite interstitielle chronique, *ibid.*, 1891, xi, 345.

fibril bundles have entirely disappeared, leaving only the discs. The nucleus of a cell at this stage is generally abnormal also. It shows irregularities in outline and an abnormal distribution of the chromatin. In very severe cases, the sarcoplasmic discs themselves tend to break down and become irregular. This was particularly noticed in a heart in which the fibrous areas had become hard and infiltrated with inorganic material. As shown in Plate XXII, Fig. 12, these cells become very small, and are markedly different from the nearly normal cell in the same figure. There are sometimes seen, as shown in Fig. 10, B, large spaces in the cell. These, however, are not constantly present, and are not a part of the process described.

Appearances met with in longitudinal section.—In longitudinal section one finds a structure, which corresponds very closely with that described in cross section. There are cells which present only peripherally disposed fibril bundles, and show a marked increase in the central perinuclear sarcoplasm. Here, also, is particularly well seen the great increase in pigment around the nucleus. As represented in Plate XXII, Fig. 13, other cells are found which show no fibril bundles at all. They are made up entirely of sarcoplasmic discs, with a considerable amount of pigment near the nucleus. The connective tissue, which surrounds these fibres, presses closely upon them, and it is sometimes difficult to say where the muscle stops, and the connective tissue begins. Muscle-cells similar to this but much smaller are also seen (Plate XXII, Fig. 14). The pigment is still abundant, and the cell itself has become spindle-shaped, owing, perhaps, to the pressure of the connective tissue. Its structure is irregular and the discs show signs of breaking up. Finally, one sees, as represented in Plate XXII, Fig. 15, long spindle-shaped spaces in the connective tissue, which contain only the muscle pigment surrounded sometimes by the remains of the cell protoplasm.

It will be seen from the above objective description, that there is in the heart-muscle of fibrous myocarditis a degeneration which runs a definite course. The normal muscle-cell, which is almost entirely filled with fibril bundles, undergoes a change which begins with those most centrally placed. The process of disintegration and solution

goes on from within out, until there is left only a single row of fibril bundles at the periphery of the cell. There are often great irregularities in the disappearance of the fibril bundles, but the general tendency is for them to disappear first in the central part of the cell. At a later stage the peripherally situated fibril bundles become small and disappear, leaving a cell which consists only of sarcoplasmic discs, or what corresponds to the so-called undifferentiated sarcoplasm. Such a cell is usually more or less rhomboidal, but, as the process goes on further, it becomes distinctly spindle-shaped. The size diminishes gradually, until finally there is nothing left but the detritus and a greatly increased amount of pigment.

Such a process as this means little in itself, but if it be considered in connection with the process of histogenesis which has been described, there seems to be a most interesting relation between them. It will be remembered that the cardiac cell appears first as a spindle-shaped structure, containing an irregular network, which tends to become more regular as the development goes on. Seen in cross section, it presents a number of discs which were spoken of as sarcoplasmic discs. Some of these break up into smaller ones, and between them there is an accumulation of the network to form fibril bundles. These fibril bundles form first at the periphery of the cell, and gradually develop toward the centre until the fibre is complete. It will be noticed that the last structures to develop are the fibril bundles at the centre of the cell, and as described above the first structures to degenerate are fibril bundles in this same central position. The degeneration goes from the centre out, while the development has occurred from the periphery in. One very marked stage in the histogenesis is that in which the cells have a single row of fibril bundle around the periphery, and exactly the same stage is observed in the degeneration. Even in the later stages of the degeneration the cells show a marked resemblance to the earlier stages of development. The earliest developmental stage shows a spindle-shaped cell with simple sarcoplasmic discs, while one of the later stages of the degeneration could be described in much the same way. In short, the process of degeneration is approximately a reversal of the developmental process. The first structures to be

formed are the last to degenerate, and the last ones to develop are the first to disappear. Although it has to do with the internal structure of a cell, this process is a striking example of a principle which seems to hold in a number of instances, namely, that the most highly differentiated tissues, or parts of an organ, tend to degenerate first.

In conclusion, I wish to thank Dr. Flexner for the kind interest which he has taken in this study, and for his many helpful suggestions. I am also indebted to Mr. Eggers of this laboratory for the care with which he has prepared photographs for me.

DESCRIPTION OF PLATES XXI AND XXII.

Plate XXI.

Fig. 1. Longitudinal section of normal adult human heart muscle. *S*, sarcoplasmic discs; *F*, fibril bundles; *K*, Krause's membrane; *A*, the junction of two sarcoplasmic discs.

Fig. 2. Transverse section of normal adult human heart muscle. *S*, sarcoplasmic discs; *C*, central sarcoplasmic mass; *F*, fibril bundles; *A*, junction of two sarcoplasmic discs. The section is through a part of the cell, either above or below the nucleus.

Fig. 3. Transverse section of heart-muscle cells from a pig embryo 10 mm. long. *S*, sarcoplasmic disc.

Fig. 4. Transverse section of heart-muscle cells from a pig embryo 20 mm. long. *F*, fibril bundles; *S*, sarcoplasmic disc.

Fig. 5. Longitudinal section of parts of two cardiac muscle cells separated by a cement line. *A* is extended, *B* contracted muscle.

Fig. 6. Longitudinal section, showing a break in the contracted muscle in *A* and a break in the extended fibre in *B*.

Fig. 7. Microphotograph of longitudinal section of heart muscle showing the degeneration of fragmentation. ×700.

Fig. 8. Longitudinal section of heart muscle showing the earlier stages of degeneration. *A*, extended muscle; *B*, degenerated area; *C*, cement line.

Fig. 9. Longitudinal section of heart muscle, presenting later stages of degeneration. The fibre *B* is broken across as a result of the degeneration.

Plate XXII.

Fig. 10. Transverse section of degenerating muscle fibres in fibrous myocarditis. *A*, cells in which the peripheral fibril bundles remain. The rest of the cell is made up of sarcoplasmic discs. *B*, a cell which shows large spaces or vacuoles.

Fig. 11. Transverse section of degenerating muscle fibres in fibrous myocarditis. The cells at the edge are normal; the central one has lost all its fibril bundles.

Fig. 12. Transverse section of an area of heart muscle infiltrated with inorganic material in a case of fibrous myocarditis. *A*, connective tissue replaced partly by inorganic material. At the left hand of the figure muscle cells are shown in various stages of degeneration.

Figs. 13, 14 and 15. Longitudinal sections of degenerating fibres in fibrous myocarditis. The fibril bundles have all disappeared. In 14 the cell has become somewhat spindle-shaped, and in 15 only the pigment granules and a suggestion of sarcoplasm remain.

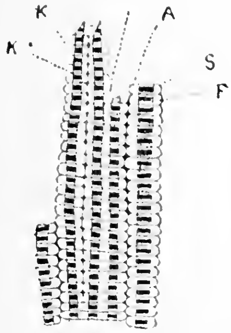


FIG. 1.

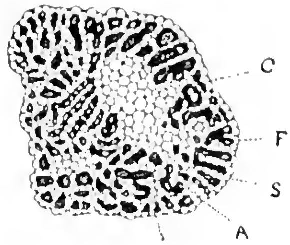


FIG. 2.

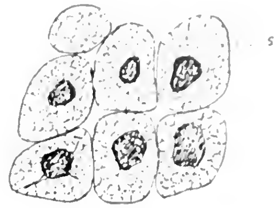
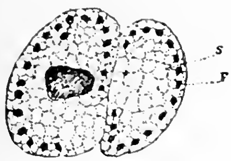


FIG. 3.



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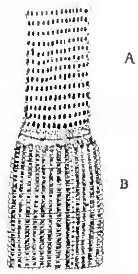

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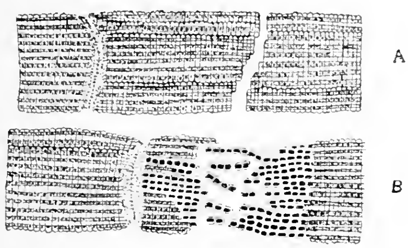

FIG. 6.

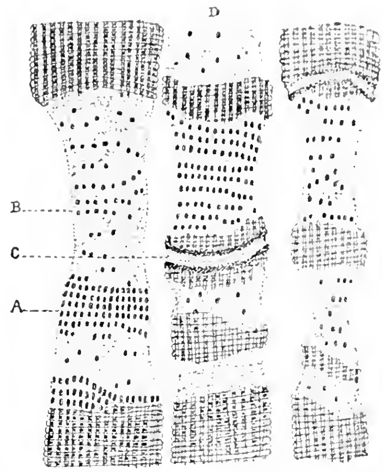

FIG. 8.

FIG. 7.

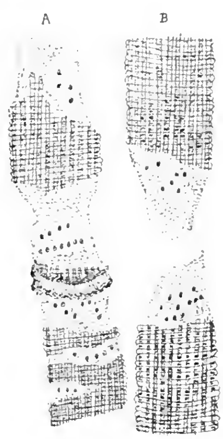

FIG. 9.

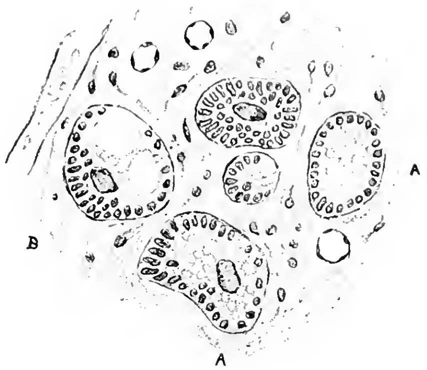

FIG. 10.

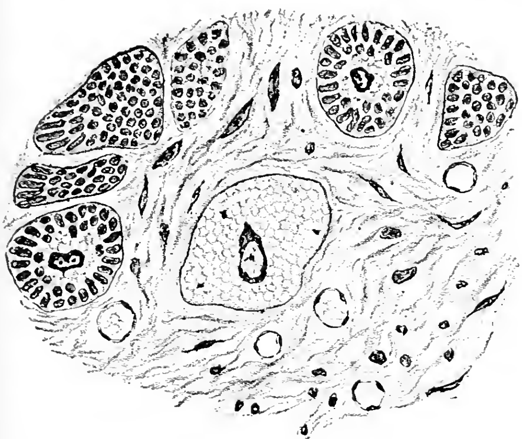
FIG. 11.

FIG. 12.

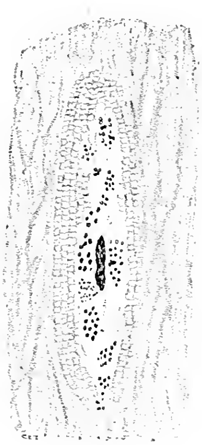
FIG. 13.

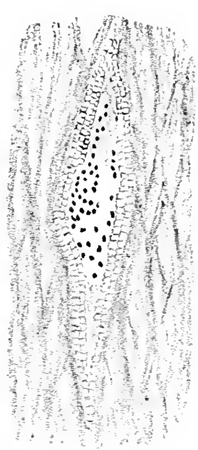
FIG. 14.

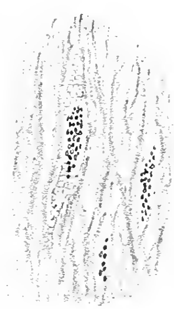
FIG. 15.

CULTURES FROM THE BLOOD IN SEPTICÆMIA, PNEUMONIA, MENINGITIS AND CHRONIC DISEASES.

By FRANKLIN WARREN WHITE, M. D.

(From the Pathological Laboratory of the Massachusetts General Hospital.)

The value and significance of bacteriological examination of the blood are so evident that many observations by this method have been made in the last twenty years in an endeavor to throw light on the etiology and course of infectious diseases. The object of such investigations is not wholly theoretical, but is also practical as a means of diagnosis, prognosis, and, possibly, treatment. The results obtained have been varied and contradictory, which is probably due in part to faulty methods. The old method of pricking the skin and using a few drops of blood for culture has the disadvantage that too small an amount of blood is used to find the bacteria if they are few in number, and that it involves great danger of contaminating the cultures with bacteria from the skin. The later method of aspiration of a superficial vein by cannula or sterile syringe has the advantage of furnishing a satisfactory amount of blood for examination and lessening the dangers of contamination. The method of Petruschky (47), namely, blood-letting by means of a wet cup, is more complicated and less satisfactory than the latter method. Direct animal inoculation with blood from a patient, as used by Petruschky and others, is not always reliable as compared with cultures on suitable media.

Until recently post-mortem findings have been considered of equal significance with intra-vital ones, but there is no question that cultures during life, in spite of incompleteness of methods, furnish a better indication of general blood invasion during disease than autopsy reports, as the latter do not exclude agonal and post-mortem invasions. Wyssokowitsch (62), as a result of his studies on the filtering of bacteria out of the blood by the liver and kidneys, stated that in

any ordinary course of infection the invading bacteria would be so quickly and completely separated from the blood that there would be very small chance of finding them by methods of blood culture. Many other observers, on the contrary, have found bacteria in the blood in a large percentage of cases examined and have probably over-estimated the significance of these results and the value of blood cultures as a means of diagnosis in infectious diseases.

Our own observations extend over a series of 92 cases, consisting of 18 cases of severe sepsis, 19 cases of lobar and lobular pneumonia due to the pneumococcus, 8 cases of epidemic cerebrospinal meningitis, 37 cases of severe chronic disease, and 10 miscellaneous fatal cases. Cultures were made during life, usually in the later stages of disease, and in many of the cases as soon as possible after death (one-half hour). The cultures in cases of septicæmia, pneumonia and meningitis were made in order to find out, if possible, how frequently, in addition to toxine absorption, the blood was invaded by the specific organism of the disease; also the time of occurrence of such general invasion, and its relation to mortality.

In the cases of chronic disease the cultures were made both before and after death to determine the frequency of general blood invasion in the late stages of disease, and during the last few hours of life—the so-called "terminal infections" and "agonal infections."

Methods.—The blood was obtained during life as follows: The skin about the elbow was carefully scrubbed with soap, water, alcohol and ether and a tight bandage was tied around the upper arm to distend the veins about the elbow. One of the large superficial veins was aspirated with a sterile glass syringe (an ordinary glass antitoxin syringe, with asbestos packing and a two-inch needle of rather small bore, was used) and 5 cc. of blood withdrawn. The aspiration was rendered practically painless by use of an ethylchloride spray; 0.5 cc. of the blood was forced directly from the syringe into each of eight tubes of agar, kept fluid at the bedside at a temperature of 42° C. The blood was thoroughly mixed with the agar and four of the tubes slanted and cooled. The other four were plated. Two bouillon tubes were each inoculated with 0.5 cc. of the blood. The plates and tubes were kept in the thermostat at body temperature and examined at intervals for several days.

Blood was obtained after death by aspiration of the heart. The skin was prepared in the same manner as during life and the same syringe used, with a longer ($3\frac{1}{2}$ inch) needle, which was thrust through the 4th costal interspace close to the sternum (to avoid the lung) into the right or left ventricle of the heart and 5 cc. of blood withdrawn and planted in cultures as just described. As the needle in this method passes through the pericardial and probably the pleural cavity on the way to the heart, blood was taken after death only in cases where these cavities were not infected. One to three specimens of blood were taken in each case. The time of taking the blood was from 10 days before to one-half hour after death in the fatal cases, or at intervals of several days during the height of disease in the cases which recovered.

The bacteria found in the cultures were identified by microscopical examination, growth on various media and a comparison with the organisms found in metastases or at autopsy. In the large number of cultures made contaminations were relatively few and easily recognized by their distribution and character of growth on the media.

I.—SEPTICÆMIA. PNEUMONIA. MENINGITIS.

Before describing our own results we will briefly review the more important previous work on these subjects. Septicæmia has furnished a fruitful field for observation.

Garré (25), Rosenbach (51), Brunner (10), and Blum (7) report single positive results of blood cultures in cases of septicæmia. A few drops of blood were taken during life by pricking the finger. Brieger (9) in six severe cases of puerperal sepsis took blood from a vein during life and obtained in each negative results. Czerniéwski (16), using blood from finger and vein, in 370 cultures from 37 cases of puerperal sepsis obtained positive results in only 15 tubes from 10 cases: all the severe cases gave pure cultures of streptococci. Sänger (52) found staphylococci in blood taken from a vein in four cases of sepsis.

Later observers urged the necessity of using larger quantities of blood (1 to 5 cc.) for cultures owing to the small number of bacteria present in the blood. The method of venous aspiration with a syringe was introduced with varying results. Blum found Staphylococcus albus in two cases of sepsis several days before death. Canon (11), in addition to positive results in 40 out of 70 cases of sepsis, pyæmia, and osteomyelitis, where blood was taken several hours after death from an arm vein, obtained 11 positive results in 17 of these cases during life, usually 2 or 3 days before death in fatal cases. He believes that bacteria are present in the blood of almost all cases of sepsis in the late stages of disease.

and grow and increase in the blood in some cases. Microli (39) found Staphylococcus pyogenes albus in each of his four cases of septicæmia during life. Parascandolo (46) examined eight cases of pyæmia and found pyogenic streptococci in blood during life in each. Hirschlaff (31) in eight cases of local and general sepsis found staphylococci or streptococci in seven. Sittmann (53) found staphylococci or streptococci in each of nine cases of septico-pyæmia from six hours to fifteen days before death, and believes that pus organisms are always present in the blood in such cases, and that blood cultures are the surest means of diagnosis. He concludes that Canon's observation that streptococci are found in the blood only a few days before death was due to faulty methods.

In contrast to these abundant positive findings other observers using larger amounts of blood and better methods have found specific bacteria present in the blood in a smaller percentage of their cases. After allowing for individual differences in patients, the suspicion of contamination in the earlier cases is inevitable. Many of the observers have not taken into consideration the demonstration by Welch (58) of the frequent, if not constant, presence in the deeper layers of the epidermis and in the glandular appendages of the skin of the white staphylococcus and of the inability to destroy this organism by ordinary methods of cutaneous disinfection. Welch has called attention to the absence of diagnostic significance attaching to the demonstration of ordinary cutaneous bacteria, particularly white staphylococci, in blood withdrawn by cutting or pricking the skin.

It is interesting to note that Petruschky (47) using a rather objectionable method, obtaining the blood for cultures by means of wet-cupping, in 59 cases of sepsis obtained only 17 positive results: streptococci 15 times, staphylococci twice. Neumann (42) in 5 cases of pyæmia, using large amounts of blood from an arm vein, obtained negative results. E. Grawitz (26) in 7 cases of endocarditis found pyogenic cocci in the blood only once. Kraus (33) in 88 cases of infectious disease (puerperal fever, endocarditis, tuberculosis) found staphylococci or streptococci in the blood in 17 cases. In a second series of 104 cases of infectious disease using a pointed bent hollow needle and taking the blood directly from the vein to the culture media, he obtained only 12 positive results; 22 of these cases were septicæmia, erysipelas and endocarditis, and these gave 7 positive results. He emphasizes the fact that positive blood cultures to be of diagnostic value must be of "specific bacteria," whose nature excludes the possibility of their being contaminations. Kühnau

(34), using 10 cc. of blood taken from a vein through a cannula directly to the culture media, in 45 cases of septicæmia and local purulent infections obtained 4 positive results, and in 76 cases of endocarditis 2 positive results. In a considerable number of cases he made cultures of blood obtained by the old method of pricking the finger, as well as by aspiration of a vein. The results obtained furnish an interesting commentary on the unreliability of the earlier method. In 23 cases of septico-pyæmia three positive results were obtained by the venous aspiration method; in 18 of these cases the finger blood was examined, in 12 staphylococci were found. In 12 cases of ulcerative endocarditis, Staphylococcus pyogenes aureus was found once in venous blood, while bacteria were found 11 times in the finger blood. Hewelke (30), in examining febrile cases of phthisis, found pus organisms in blood from the finger in 14 cases out of 27, and 3 times out of 27 where the blood was taken by puncture of a vein.

In contradiction to the belief of these last-named authors, that even in severe septic cases only a relatively small number of bacteria circulate in the blood, is the statement of Nocard (43) that directly after taking food an abundant passage of bacteria occurs from the intestinal canal to the blood by means of the chyle. This was confirmed by Desoubry and Porcher (17) in experiments upon dogs. A number of clinicians have considered the intestine the starting point of bacteria in various infections through the blood. Tavel (55) in strumitis, A. Czerny (15) in certain skin diseases, Posner and Lewin (48) in "cryptogenetic septicæmia," and Fischl (18) in septicæmia in infants. The passage of bacteria into the blood by means of the chyle has been disproved, however, by the later work of Neisser, Kühnau and others. Neisser (40) investigated the chyle of large dogs by tying a cannula in the thoracic duct and found it uniformly sterile even after feeding the dogs abundantly with bacteria. Kühnau obtained a similar result in five dogs. Neisser found the mesenteric glands of large animals sterile. Fodor (20) has shown that the blood of normal animals is sterile and Meissner (37) and Hauser (28) that the organs are sterile.

Blood cultures made in cases of pneumonia by several authors indicate that a general blood invasion by the pneumococcus occasionally occurs during life. Belfanti (4) in "many" cases (number not given) obtained 6 positive results; five of these proved fatal. Boulay (8) of four cases found pneumococci in two a few hours after death. Friedländer (23) in 6 cases had one positive result. Sittmann (53) in 16 cases obtained 6 positive results (in 2 of which pneumococci were found only

in stained specimens of blood, not in cultures). Of the 18 negative cases 2 died; of the 6 positive cases, 4 died. The positive results were obtained from one to seventeen days before death in the fatal cases. Kraus (33) in 12 severe cases of pneumonia found the pneumococcus once, one day before death; of 11 negative cases 10 recovered. Kohn (32) in 32 cases found pneumococci in the blood of 9; 7 of the positive cases died and 2 recovered after metastatic pneumococcus infections; of 13 negative cases, 8 recovered. Excluding two cases dying of complications, we find that a considerable majority of the negative cases recovered and of the positive cases died. He concludes that the presence of pneumococci in the blood gives a very unfavorable prognosis. The positive results were obtained only 24 to 48 hours before death in the fatal cases. Kühnau in 9 severe cases found pneumococci in two cases, both fatal. Another fatal case was negative.

An epidemic of cerebrospinal meningitis was in progress at the time of our blood investigation and cultures were made in eight cases. Weichselbaum (57), Netter (41), Heubner (29), Councilman (14) and others have studied the disease and do not find the specific organism, Diplococcus intracellularis, at autopsy, except in connection with the lesions of the disease, and conclude from post-mortem cultures of the blood, liver, spleen, and kidneys that it never produces septicæmia. The abdominal and thoracic organs are frequently found sterile at autopsy.*

My own observations embrace 18 cases of severe *sepsis* (7 appendicitis with general peritonitis, 2 phlegmon of leg, 2 septic wounds, 1 osteomyelitis, 1 suppurative periostitis, 1 suppurative nephritis, 1 facial erysipelas, 1 abscess of the appendix, 1 empyema), all of which were fatal, and eight of which were autopsied. They are chiefly cases of severe local septic infection without formation of metastatic abscesses, only one case being pyæmic. In the 18 cases, 37 blood cultures were made and specific bacteria were found in the blood during life only four times—Streptococcus pyogenes in pure culture three times and Staphylococcus pyogenes aureus in pure culture once. We give a brief description of the four positive cases:

* Since the completion of this article Gwyn (*Bulletin of the Johns Hopkins Hospital*, 1899, x, 112) has reported a case of epidemic cerebrospinal meningitis in which during life he obtained in pure culture Diplococcus intracellularis meningitidis, not only from fluid withdrawn by lumbar puncture, but also from the blood and from the fluid aspirated from an inflamed joint.

Case I.—Phlegmon of both legs following amputation of feet. Culture from leg showed Streptococcus pyogenes. Temperature 101° to 105° F.; death in 9 days. Blood culture 6 days before death, negative; 4 days before death, 40 to 60 colonies of Streptococcus pyogenes per cubic centimetre. No autopsy.

Case II.—Suppurative nephritis. Renal symptoms for 4 weeks; pyuria. Temperature 102° to 103° F.; death. Blood culture 3 days before death, 2 to 15 colonies of Staphylococcus pyogenes aureus per cubic centimetre. Autopsy, 58 hours after death, showed prostatic hypertrophy, cystitis, pyelonephritis, ureteritis, acute pleuritis, abscess of lung. Cultures from heart, liver, kidney, spleen, lung, ureter showed Staphylococcus pyog. aureus.

Case III.—Phlegmon of arm. Culture showed Streptococ. pyog. Temperature 100° to 103° F.; cervical adenitis; death after 2 weeks. Blood cultures 10 days before death, negative; 2 days before death 50 to 60 colonies of Streptococ. pyog. per cubic centimetre; one-half hour after death 1200 to 1500 streptococci per cc. No autopsy.

Case IV.—Erysipelas. Acute intestinal obstruction, laparotomy. One week later facial erysipelas, local peritonitis and otitis media. Temperature 103° to 104°; death. Blood cultures 5 days before death, negative; 2 days before death, 15 to 20 colonies of Streptococ. pyog. per cc.; three-quarters of an hour after death 20,000 Streptococ. pyog. per cc. Autopsy 13 hours after death showed malignant adenoma of sigmoid flexure, circumscribed peritonitis with multiple abscess formation, double purulent otitis media. Cultures from scalp, ear, peritoneum, liver, showed Streptococ. pyog.; spleen sterile.

In each of the foregoing cases the species of bacteria causing the initial lesion produced also the general invasion, no heterologous organisms being found. In the two positive cases in which an autopsy was performed the bacteria found in the blood during life were also found distributed through the organs at autopsy. The blood cultures in the other 14 cases were negative, in two cases one or two cultures being contaminated with cocci from the skin or air. In the 6 negative cases in which an autopsy was performed, while scattered germs were found in certain of the organs, there was no evidence of a general infection. These facts speak well for the method of blood culture employed in detecting a general bacterial invasion when it occurs.

The number of bacteria found per cubic centimetre during life

was never large, at most 50 to 60 streptococci. In two cases (III and IV) there was a great increase in the number of bacteria found immediately after death over the number found two days before. This may be interpreted to mean either that a growth of bacteria occurred in the blood during the last two days of life, or that a largely increased number were able to enter the blood during the last days or hours of life as a result of diminished body resistance.

The time of bacterial invasion of the blood was late in the disease, cultures from the fifth to tenth day in three of the subsequently positive cases being negative. The probable explanation of this is that the bacteria did not succeed in invading the blood till the body resistance was much reduced, this general spread and growth of bacteria, accompanied by an increased production of toxines, together with the lowered body resistance, leading speedily to a fatal ending.

In 7 cases in which the blood was negative before death it was examined also after death. The cultures were negative in each of these instances. An autopsy was performed in 6 of these cases and showed absence of any general invasion and several sterile organs at each autopsy. No general agonal invasion occurred in any of these patients.

Our 19 cases of *lobar pneumonia* were all at least moderately severe, and 10 were fatal. Of the latter 9 were autopsied. Thirty-two cultures were made, and in three fatal cases Diplococcus pneumoniæ was obtained from the blood during life. We will briefly describe the 3 positive cases:

Case V.—Lobar pneumonia. Temperature 103° to 104°; delirium; death. Blood cultures, 3 days before death, negative; one day before death, 40 to 60 colonies of the pneumococcus per cc. Autopsy 2½ hours after death: consolidation of right lower and middle lobes, right pleuritis with effusion. Cultures from liver, lung, heart and spleen showed pneumococci; kidney sterile.

Case VI.—Acute bronchopneumonia. Duration 2 weeks; temperature 100° to 101°; cough, dyspnœa, orthopnœa; slight œdema of ankles; vomiting; three general convulsions, death. Blood cultures 4 days before death negative; 2 days before death, 18 to 30 pneumococci per cc. Autopsy 3 hours after death: acute bronchopneumonia, acute fibrinous

pericarditis and peritonitis, acute glomerulonephritis. Cultures from lung, heart, pericardium, peritoneum, spleen, liver and kidney showed pneumococci.

Case VII.—Lobar pneumonia. Duration 8 days; temperature 101° to 103°; delirium; death. Blood cultures 5 days before death, negative; 2 days before death, 10 to 15 pneumococci per cc.

In two of the positive cases at autopsy a general pneumococcus infection was found; in the third case no autopsy was performed. In the 8 negative cases in which autopsy was performed, pneumococci were found in the lung, but there was no evidence of a general infection. No organism save the pneumococcus was found in the blood in any case. The number of bacteria was not large, from 10 to 60 per cc. The time of general infection was always late in the disease, negative results being obtained on the 3d to 5th day before death, and positive results one to two days before death.

Our 8 cases of *cerebrospinal meningitis* were all severe and 6 of them fatal. An autopsy was performed in 4 cases and diplococci found in the meninges; in one case they were identified as Diplococcus intracellularis meningitis. No pathogenic bacteria were found in the blood cultures. In 5 cases blood cultures were made shortly after death, and all proved sterile. The thoracic and abdominal viscera were found sterile in three out of four autopsies. In the other case (No. II), a culture shortly after death was sterile, while at autopsy miscellaneous bacilli and cocci were found in the organs. Their presence was probably the result of post-mortem invasion.

In reviewing our cases with reference to frequency of invasion, it is seen that our results in septicæmia are in accord with those of later observers, such as Neumann, Kraus, and Kühnau; we have not obtained the frequent positive results of the earlier investigators. Our series of fatal septic affections proved to be, for the most part, cases of intoxication, with resorption of toxines produced by bacteria growing in a local primary focus, and only in a small proportion of cases did the organisms enter the general circulation. The frequency of general invasion in our pneumonias is similar to that in the cases of Kohn, Kühnau, and Kraus.

Regarding the relation of general infection to mortality, no conclusion can be drawn from our septic cases, save that a large percentage may die without general infection. On the other hand, several authors have found bacteria in the blood in cases of septicæmia which ultimately recovered. Bernheim (5), in 2 such cases, found streptococci; Sittmann, in 5 cases, found staphylococci; Petruschky, in 8 cases, found streptococci of " high virulence." All our cases of pneumonia with positive blood cultures died, while 9 negative cases recovered and 8 negative cases died; or, to put it in another way, a general invasion was found in less than one-quarter of the fatal cases. Ordinarily, pneumococci have been found only in the severest cases, but Sittmann and Kohn each report two instances, and Belfanti one, where recovery followed a positive blood culture.

I believe that the value of blood cultures as a means of diagnosis in obscure cases of so-called " cryptogenetic sepsis " has been overestimated. Positive results during life are always interesting and valuable, and, when secured by proper methods, are removed from the suspicion of agonal or post-mortem invasion which sometimes obscures autopsy findings, but it is evident, from the large percentage of negative results even in the severest types of disease, that the search for the specific causes of disease by this method will often prove futile. As regards prognosis, it is evident that a negative culture does not give much assistance, while a positive result gives a very unfavorable prognosis in the majority of cases. We have never noted any marked change in the clinical course coincident with the occurrence of a general infection.

In our patients the time of general infection in both septicæmia and pneumonia has been late in the disease, only a few days before death. This has been the experience of Canon, Czerniéwski and Kühnau in cases of septicæmia, and of Kohn in cases of pneumonia. This, in my opinion, is either because the occurrence of a general infection led speedily to a fatal termination, or because the general infection was in itself an index of weakened body resistance and general breakdown. Sittmann, on the contrary, has obtained positive results in sepsis from 5 to 25 days before death, and found the pneu-

mococcus in the blood in pneumonia from 1 to 18 days before death. (Compare also the positive cases of blood culture followed by recovery mentioned above). The length of life after general infection depends on the virulence of the germ and the degree of body reaction, and it is evidently possible that the body may eliminate all germs from the blood and recover, or may eliminate a part and then succumb, as is seen in A. Fränkel's (21) case where 200 pneumococci per cc. were found in the blood four days before death and a much smaller number two days before death. Wyssokowitsch, in his experiments of injecting cultures of various bacteria into the veins of animals, found that the bacteria disappeared more or less completely from the blood after injection and were deposited in the liver, spleen and bone marrow, where the non-pathogenic bacteria were killed off as a rule, while the pathogenic bacteria increased and re-entered the blood.

I wish to say a word concerning the mode and cause of general blood infection. Kraus, in an article upon the resorption of micro-organisms into the blood from various organs, as the lung, intestine, bladder, tonsils and gall-bladder, after a careful review of the literature, concludes that in general the organs of the body are permeable for bacteria, some under normal conditions, some after damaging of tissue, and from all these organs with greater or less difficulty a resorption infection of the blood may occur. The exact factors concerned in producing a lessened body resistance, an increased susceptibility, are not known, but undoubtedly the chief defences against the invasion of pathogenic micro-organisms are the body fluids and cells. It thus becomes of great interest to know whether the normal blood is destructive to pyogenic organisms and loses its germicidal power under conditions which predispose to a general infection. It was my intention to test the germicidal power of the blood for pyogenic cocci in cases of septicæmia in order to determine whether general invasion could be traced to a loss of germicidal power. A review of the literature showed that this property of the blood varies in man and animals, both among themselves and for different species of bacteria. Nuttall (44), Stern (54) and Prudden (49) have concluded from their experiments with human blood and normal body fluids that they

possessed little or no effect upon pyogenic cocci. A series of experiments by the author (60), which were recently published, confirm this belief that normal human blood serum is not actively germicidal for pus organisms. Thus it is evident that there are factors other than the germicidal power of the blood which play an important part in protecting the body against general infection and that the solution of this problem must be sought along other lines.

II.—CHRONIC DISEASES.

It is a well-known fact that local infectious processes are of frequent occurrence in patients afflicted with chronic diseases, and autopsy findings indicate that general infection occasionally occurs as a cause of death. Believing that in many cases results obtained during life have more significance than those obtained at autopsy, we have used the method of blood cultures during life to determine the frequency of general terminal infections. Osler (44) says: "It may seem paradoxical, but there is truth in the statement that persons rarely die of the disease with which they suffer. Secondary infection, or, as we are apt to call them in hospital wards, terminal infections carry off many of the incurable cases in the wards." Flexner (19), in an analysis of the autopsy reports of 225 cases in which occurred chronic cardiac, vascular or renal disease, alone or in combination, found 213 cases in which bacteriological examination gave positive results. Local infections were found in a large proportion of all his cases of chronic nephritis, arteriosclerosis, hepatic cirrhosis and other chronic diseases. Acute infections of the pericardium, pleura, peritoneum, meninges and endocardium were most frequent. In 163 cases of chronic nephritis, either single or combined with other chronic disease, 38 cases were found with a general distribution of bacteria in the organs. In 63 cases of cardiac and arterial disease he found 14 similar cases. These were considered instances of general infection during life, and in many a local lesion was present, such as erysipelas or peritonitis, which was looked upon as the source of the general infection. In a large proportion of the cases, visible focal lesions were not present in the organs at autopsy. The pyogenic cocci were

usually the infectious agents. He believes this susceptibility to infection in chronic disease to be a result of changes in the blood occurring in cachexiæ, weakened body resistance being due to a loss of germicidal power of the serum. These are very interesting and significant results, yet it is only fair to say that a general distribution of bacteria at autopsy does not always mean general infection during life. If all the cases could be excluded where the bacterial invasion of the blood and tissues may have occurred not during life, but during the death agony or after death, the list of "general infections" would undoubtedly be smaller.

Of particular interest also are the general blood infections, which have been demonstrated during life in chronic diseases by means of blood cultures. Petruschky examined the blood in 8 cases of advanced pulmonary tuberculosis and found streptococci present once during life. In 8 of 14 cases which came to autopsy, streptococci were found in all the organs. Sittmann obtained positive results in 3 of 4 cases of phthisis, finding Staphylococcus pyogenes aureus twice in the blood and Staphylococcus pyogenes albus once. The number of bacteria was only 2 or 3 per cc., and they were found from 2 to 30 days before death. Hewelke, in 27 cases of phthisis, found pyogenic cocci in the venous blood in 3 cases. Michaelis and Meyer (38), in examining the blood in 10 cases of phthisis, found pyogenic cocci in 8 cases from 2 to 9 days before death. Hirschlaff (31) obtained staphylococci from the blood in 4 out of 25 cases of phthisis. Fränkel (22) found colon bacilli in the blood during life in a case of leukæmia. Gabbi and Barbacci (24), in 2 cases of pseudo-leukæmia, obtained the same result. Verdelli (56), in 2 cases of pseudo-leukæmia, found Staphylococcus pyogenes aureus and albus. As an analogue to this class of cases, many observers have demonstrated the occurrence of secondary infections by pyogenic cocci in cases of acute disease, such as typhoid fever and diphtheria.

Turning from this subject for a short time, I wish to speak of another which is rather closely allied to it and upon which we have also made some observations. There is a certain amount of evidence drawn from blood cultures made at the time of death, from the bac-

teriological examinations at autopsies, and from experiments upon animals, that during the last few hours of life the bacteria which are present in certain organs, more especially the bowel, are able to overcome the weakened powers of resistance of the individual and to get into the blood stream, and are distributed by means of the circulation to the various organs, and may grow in these organs after death. This process, which is termed "agonal invasion," naturally has no influence on the course of disease in the individual, it being a result rather than a cause of disease, but it has a very important bearing on the value and significance of positive bacteriological findings at autopsy. If an "agonal invasion" of bacteria is a frequent occurrence, it is very evident that the bacterial contents of organs at autopsy do not represent the conditions present during life; that cultures alone are not sufficient to put the organism found in causal relation with the pathological changes present, and that our conclusions as to the causes of disease from post-mortem bacteriological findings must be much restricted. To throw light on this subject of agonal invasion we have made blood cultures before and after death in our chronic cases, and in a series of miscellaneous fatal cases, and post-mortem cultures in some cases of septicæmia and meningitis already referred to. We will give a brief summary of the more important literature before speaking of our own results.

The first observations of the general invasion of internal organs of the body by the colon bacillus were made by Welch (59) and reported in 1890. He reports that this organism was found in 33 autopsies out of about 200 at the Johns Hopkins Hospital, with especial frequency in cases with lesions of the intestinal mucosa. He regards it as in most instances a harmless invader without influence upon the course of the disease and without pathogenic effects. Many observers have since noted the frequency of occurrence of the colon bacillus in organs at autopsy. Intestinal bacteria have been sometimes reported as present in the blood very shortly after death. Beco (3) in studying the bodies of patients who had died of chronic disease found the colon bacillus present immediately after death in the liver in 11 cases and in the heart in one case; also the colon bacillus and Staphylococcus pyogenes aureus together in the liver in 3 cases. Létienne (36) found the colon bacillus in

the gall-bladder 11 times in 18 cases, in 3 cases 15 minutes after death. Achard and Phulpin (1) obtained blood from the veins and liver during the death agony, and also made cultures at autopsy in 13 chronic cases. In no cases were bacteria found in the blood before death, in 8 cases bacteria were found in the liver from 10 minutes to 10 hours ante mortem (B. coli 6 cases, Staph. pyog. aureus and Staph. pyog. alb., each one case). In 24 cases no bacteria were found before death, but Staph. pyog. aur., colon bacillus and putrefactive bacteria were found in the organs at autopsy. In 11 cases no bacteria were found either before death or at autopsy. They conclude that agonal invasion occurs, but is rather rare and that the intestine is the chief source of the germs. Hanot (27) in a case of jaundice, obtained a culture of colon bacilli from the liver during life.

Wurtz and Herman (61) froze small animals to death and obtained cultures of intestinal germs from the organs during the death agony in 21 out of 33 cases, while control animals rapidly killed remained sterile. Other animals poisoned with arsenic or killed by asphyxiation gave similar positive results. Beco killed rabbits slowly by poisoning with tartar emetic and cantharides and found intestinal germs present in the organs immediately after death in most of the cases, while animals rapidly killed remained sterile. His method of culture by means of bouillon tubes is objectionable. Chvostek and Egger (13), repeating Wurtz and Herman's work, froze 13 animals and obtained positive results in 30 per cent of the cases, while control animals were all negative. Later they froze 50 animals, and examination of the heart's blood immediately after death gave 44 per cent of positive results. Another series of animals frozen and similarly examined two hours after death gave 16 per cent of positive results. He explains this lower result by saying that the germs which invaded the blood were partly killed off by the serum on standing two hours. Starved animals gave negative results. Animals which were stabbed gave 20 per cent positive results, which can hardly be due to agonal invasion and are not explained. In all these experiments the peritoneum contained more bacteria than the blood.

Chvostek (12), in reviewing his own work and that of Wurtz and Herman, Beco, Achard and Phulpin, and others, concludes that bacterial invasion of the body during the death agony occurs frequently, that what has been considered post-mortem invasion is really the result of agonal invasion and post-mortem growth. At the end of life the vigorous germs, usually the pyogenic cocci which can overcome live cells, invade the body first, then the weaker ones which can overcome damaged cells,

and last the putrefactive germs. The more cachectic the individual the more easily agonal invasion occurs. The anatomically unaltered vessel walls have been shown to be permeable for bacteria, and Chvostek believes there may be a procession of bacteria into the blood, some being killed off while others are steadily invading the circulation. He believes that bacteriological findings at autopsy are not a safe ground for conclusion about the conditions which were present in life.

On the other hand, while admitting that intestinal germs may reach certain abdominal organs during the last hours of life, there is a good deal of evidence against the frequent occurrence of an agonal invasion and general distribution of bacteria over the body by means of the circulation. Sterile organs at autopsy are a very common occurrence in all sorts of non-infectious diseases, and even in infectious cases apart from the specific foci. Lesage and Macaigne (35) examining cadavers in winter found many of them sterile. Austerlitz and Landsteiner (2) and Beco remark upon the infrequency of finding the colon bacillus in fresh cadavers. On examining the results of Achard and Phulpin, we see that in the 8 chronic cases in which agonal invasion was believed to have occurred, the heart's blood was found sterile in all as late as 2 to 23 hours after death. In 24 other cases where the heart's blood was examined from 2 to 16 hours after death, only 2 positive results were obtained, both 10 hours and later after death.

Hauser, by injecting cultures of bacteria into the dead bodies of men and animals immediately after death, has shown that a rapid and widespread invasion of the body can occur in the period of time which intervenes ordinarily between death and autopsy. In his experiments the spread of bacteria was dependent partly on the position of the cadaver; if the animal was hung up by the hind legs, the liver, pleura and heart gave positive results, and if hung up by the fore legs, the bacteria were found in the liver, kidney and bladder. In experiments upon human cadavers, the pleura, liver, kidney and bladder were invaded after intraperitoneal injection of germs, and the pericardium and heart usually remained sterile. In a large number of autopsies performed 10 to 24 hours post mortem, he found the colon bacillus present in nearly half. Positive results were much more abundant in warm than in cold weather. He believes they are due largely to post-mortem invasion of the body.

With reference to the entrance of bacteria from the intestine into the blood, the statement of Nocard that the chyle acts as a vehicle for germs has been disproved by Neisser and Kühnau. They found the chyle and mesenteric glands of large animals uniformly sterile. Wyssokowitsch

found that in dogs and rabbits, when large numbers of Staphylococcus pyogenes aureus had been brought into the gut, either by the mouth or by direct injection, the mesenteric glands on later examination were found sterile in the great majority of cases; in a few cases in which contamination could not be excluded, a few bacteria were found. Heubner has shown that there are no bacteria in the wall of the gut in infants with intestinal disease. Ribbert (50) and Bizzozero (6) found bacteria in the intestinal wall only in the follicles of the cæcum of rabbits and in no other animals. Austerlitz and Landsteiner repeated Wurtz's and Chvostek's experiments with improved technique and different results. Fifty mice were killed by freezing and 250 cultures of the heart's blood were all sterile. Negative results were also obtained in animals slowly poisoned by arsenic. Max Neisser fed rabbits, mice and guinea-pigs freely with pyogenic cocci and typhoid bacilli, after damaging the intestine with a previous diet of broken glass and sodium fluoride: in 21 of the animals the organs were sterile at autopsy, in some others positive results were obtained which he thinks may be explained by unavoidable contaminations. In an investigation where so much depends on the technique of the removal of organs and culture-making, and the dangers of infection by manipulation are so great, negative results are more valuable than positive ones. It is evident that highly pathogenic bacteria may be present in the gut without producing general infection, and that even severe damage to the gut is not in itself sufficient to open a way for them into the circulation. Neisser concludes that there is hardly a greater danger of general infection from the bowel than from the skin or from other mucosæ.

Our own observations upon terminal and agonal infections cover 37 cases of severe chronic disease (11 cardiac disease, 9 cancer, 4 sarcoma, 3 phthisis, 3 chronic nephritis, 1 Pott's disease and nephritis, 1 arterio-sclerosis, 1 tubercular meningitis, 1 tubercular peritonitis, 1 gastric ulcer, 1 chronic rheumatism, 1 pernicious anæmia), of which 30 died in the hospital and 19 were autopsied; also 10 miscellaneous fatal cases, 5 of which were autopsied. We also include 7 cases of sepsis and 5 of cerebro-spinal meningitis, already referred to, which were negative in life, and in which post-mortem cultures were made. 79 blood cultures were made in all; 41 in the late stages of disease, and 38 one-half hour after death.

We give a brief description of the 9 cases in which blood cultures gave positive results:

Case VIII.—Chronic parenchymatous nephritis. Duration of symptoms one year. uræmia, death. Blood culture one day before death, 30 to 50 colonies of Streptoc. pyog. per cc.; one-half hour after death, 100 to 150 Streptoc. pyog. per cc. Autopsy, 17 hours after death, showed parenchymatous nephritis, anasarca, passive congestion of lungs, liver and spleen, bronchopneumonia. Cultures from heart, liver, spleen and kidney showed Streptoc. pyog.

Case IX.—Chronic parenchymatous nephritis. Duration 2 years. Temperature 96° to 97° for a week before death, abdominal pain one day. Blood cultures 2 days before death, 70 to 90 Streptoc. pyog. per cc.; one-half hour after death, 300 to 500 Streptoc. pyog. Autopsy, 15 hours after death, showed parenchymatous nephritis, anasarca, syphilitic hepatitis and orchitis, degeneration of spleen, liver and kidney, acute general peritonitis and acute pleuritis. Cultures from heart, spleen, liver, kidney, pleura and peritoneum showed Streptoc. pyog.

Case X.—Pott's disease, chronic nephritis. Duration of Pott's disease 5 years, of nephritis 1 year; exacerbation of nephritis, persistent vomiting, death. Temperature 101° for 2 days before death. Blood culture 4 days before death, 10 to 15 Streptococ. pyog. per cc.; one-half hour after death, 10 to 15 Streptococ. pyog. per cc. Autopsy, 6 hours after death, showed chronic diffuse nephritis, tuberculosis of lumbar vertebræ and lungs, tubercular salpingitis, amyloid liver, spleen and kidney. Cultures from heart, liver, spleen and kidney showed Streptococ. pyog.

Case XI.—Gastric ulcer. Gastric pain 2 months, hæmatemesis, bloody stools for 6 days, death. Temperature 102° last 12 hours. Blood culture $1\frac{1}{2}$ days before death, 6 to 10 Staphlococ. pyog. aureus per cc.; $\frac{1}{2}$ hour after death, 15 to 20 Staphylococ. pyog. aureus. No autopsy.

Case XII.—Myocarditis, pericarditis. Pericarditis with effusion 11 days, temperature 100° to 102°; death. Blood cultures 4 days before death, 5 to 8 Staphylococ. pyog. aureus per cc. Autopsy, 27 hours after death, showed fatty myocarditis, fluid in cavities, passive congestion of organs, subacute fibrinopurulent pericarditis. Cultures from liver, kidney, spleen and pericardium showed Staphylococ. pyog. aureus.

Case XIII.—Mitral and aortic stenosis. Duration of symptoms 11 months, temperature 100° to 102° for 2 days before death. Blood culture 4 days before death, negative; $\frac{1}{2}$ hour after death, 5 to 6 Staphyloc. pyog. aureus per cc. Autopsy, 17 hours after death, showed mitral and aortic stenosis, chronic passive congestion of organs, acute bronchopneu-

monia. Cultures from liver and kidney showed Staph. pyog. aureus; from lung, Staph. pyog. aureus and pneumococcus; spleen sterile.

Case XIV.—Mitral regurgitation. Duration of symptoms 6 months, death sudden, no fever or complications. Blood cultures 3 days before death, negative; $\frac{1}{2}$ hour after death, 10 to 12 Staph. pyog. aureus per cc. No autopsy.

Case XV.—Cancer of epiglottis. Duration 1 year; laryngectomy, sudden death 10 hours later. Blood cultures 2 days before death, negative; $\frac{3}{4}$ of an hour after death, 10 to 20 Streptoc. pyog. per cc. No autopsy.

Case XVI.—Compound depressed fracture of skull. Operation, death after 6 hours. Blood culture $\frac{1}{2}$ hour after death, 20 Streptoc. pyog. per cc.

In the accompanying table we have classified the total number of cases examined, the number of positive results, the time the earliest positive cultures were obtained, and the number and kinds of bacteria found.

In 5 cases of chronic disease, bacteria were found in the blood one or more days before death, as follows:

2 cases of chronic nephritis,	Streptoc. pyog.,
1 case of Pott's disease and chronic nephritis,	Streptoc. pyog.,
1 case of gastric ulcer,	Staph. pyog. aureus,
1 case of myocarditis and pericarditis.	Staph. pyog. aureus.

These are considered cases of general terminal infection. In each of these 5 patients the invasion of the blood as shown by our cultures was followed in a few days by death. Degenerative changes were usually present in the organs at autopsy, but in no instance pyæmic foci. The sources of infection could not be absolutely determined. In one case a tubercular process in the lungs antedated general infection; in another a fibrino-purulent pericarditis. In one case a broncho-pneumonia was present, in another an acute peritonitis and pleuritis, but these are looked upon as part of the general infections. The clinical aspects of these cases were not remarkable; in three there was a moderate febrile reaction a few days before death, and in one for a period of eleven days; in another the temperature was subnormal for a week before death. There was grave constitutional disturbance

in each case, but any symptoms which may have occurred as a result of general infection were obscured by those which resulted from the chronic processes. In short, these cases of terminal septicæmia could hardly have been recognized clinically without blood cultures.

I.—Positive Blood Cultures during Life.

Disease.	Number of positive results.	Time of earliest positive culture.	Bacteria found.	Number of bacteria per cc. of blood.	Number of cases investigated
		(Days before death.)			
Septicæmia	4				18
Case 1		4 days.	Streptoc. pyog.	40 to 60	
" 2		3 "	Staph. pyog. aureus	11 to 15	
" 3		2 "	Streptoc. pyog.	50 to 1500	
" 4		2 "	" "	15 to 20,000	
Pneumonia	3				19
Case 5		1 day.	Pneumococcus.	40 to 60	
" 6		2 days.	"	18 to 30	
" 7		2 "	"	10 to 15	
C.-S. Meningitis	0				8
Chronic Nephritis	2				37 (chronic cases).
Case 8		1 day.	Streptoc. pyog.	30 to 150	
" 9		2 days.	" "	70 to 500	
Chronic Nephritis and Tuberculosis	1				
Case 10		4 days.	" "	10 to 15	
Gastric Ulcer	1				
Case 11		1½ "	Staph. pyog. aureus	6 to 20	
Myocarditis and Pericarditis	1				
Case 12		4 "	" " "	5 to 8	
Miscellaneous cases	0				10
	12				92

II.—Positive Blood Cultures after Death in Cases where the Blood was Negative during Life.

		(Hours after death.)			
Septicæmia	0				7
C.-S. Meningitis	0				5
Mitral and Aortic Disease	1				16 (chronic cases).
Case 13		½ hour.	Staph. pyog. aureus	5 to 6	
Mitral Disease	1				
Case 14		½ hour.	" " "	10 to 12	
Cancer of Epiglottis	1				
Case 15		¾ hour.	Streptoc. pyog.	10 to 20	7 (miscellaneous cases).
Comp. Fracture of Skull	1				
Case 16		½ hour.	" "	20	
	4				35

In addition to the general infections in chronic cases, local infections have been frequently found at autopsy, such as broncho-pneumonia, pleuritis, nephritis, etc.; of these, broncho-pneumonia being far the most common.

With reference to the occurrence of general blood invasion during the death agony, cultures of the heart's blood were made in 35 cases where no general infection was found during life. The cultures were made as soon as possible after death, within half an hour in all but a few cases. These cases consist of 7 septicæmias, 5 cases of cerebrospinal meningitis, 16 of chronic disease and 7 of miscellaneous acute diseases. We are struck by the fact that in these 35 cases, only 4 positive results were obtained; in 3 cases examination of the blood 2 to 4 days before death had given a negative result, and in the fourth case death occurred six hours after an accident in a healthy man.

1 case of mitral and aortic stenosis,	Staph. pyog. aureus,
1 case of mitral regurgitation,	Staph. pyog. aureus,
1 case of cancer of epiglottis,	Streptoc. pyog.
1 case of compound depressed fracture of the skull.	Streptoc. pyog.

These are considered cases of probable agonal infection. In chronic cases with grave cachexia, blood invasion seems a natural event: with reference to the case of fracture of the skull we quote Hauser, who says that severe disturbance of the nervous centres favors agonal invasion. We are satisfied that the large number of negative results represent the conditions which were actually present; that if any considerable number of bacteria have invaded the general circulation in the death agony, some would have been present in the heart's blood and have been found in the 5 cc. of blood aspirated and used for cultures.

Only two varieties of bacteria occurred, and these never in mixed infection. The colon bacillus was not present in a single culture. The number of bacteria was small—as a rule, 5 to 10 per cc. of the staphylococci, and 20 to 90 streptococci per cc. In 4 of the chronic cases positive cultures were obtained, both before and after death, and in 2 of these latter there was apparently a growth of bacteria in the blood, or an increased invasion of the blood; for the second cul-

ture showed the presence of a considerably larger number of bacteria than did the first. In case VIII, chronic nephritis, a blood culture a day before death showed 30 to 50 streptococci per cc.; a culture immediately after death, 100 to 150 per cc. In Case IX, chronic nephritis, a culture 2 days before death showed 70 to 90 streptococci, and a culture immediately after death, 300 to 500 per cc. In the two other cases the number of bacteria remained practically stationary. In a large majority of our chronic cases, the blood both before and immediately after death proved sterile.

In 5 of our nine positive cases an autopsy was performed and the same bacteria which were present in the blood cultures were found distributed through the organs, as would be expected.

In many of the cases where the blood was negative during life, bacteria, such as the colon bacillus, pneumococcus or pyogenic cocci, have been found in one or more organs at autopsy. Bearing in mind Hauser's proof of the rapidity of post-mortem extension of bacteria through the body, we believe that this is the explanation of their presence in most cases; on the other hand, some probably represent local infections in life, and some agonal infections. In a considerable number of the local infections the bacteria no doubt reached the infected organ by means of the blood stream, even in cases which gave negative blood cultures during life; probably a few bacteria gained entrance to the blood-vessels, were carried about and deposited in various organs, most of them being destroyed, while a few succeeded in gaining a footing and produced a local infectious process. In such cases we could hardly expect to obtain positive results in our blood cultures.

We have reached the conclusion that general agonal invasion by bacteria is a rather uncommon occurrence, from a consideration of the frequency of sterile organs at fresh autopsies, from the uniformly negative results obtained by Austerlitz and Landsteiner, and Neisser in animal experiment, and finally from the series of negative post-mortem cultures in our own cases. We cannot accept the theory that the normal or nearly normal bowel is easily and frequently penetrated by bacteria, or the application of this theory to post-mortem findings.

If autopsies are performed within a short time after death and the results of post-mortem growth of bacteria in the body thus avoided, we have every reason to believe that in the majority of cases the bacteriological findings at autopsy correspond to conditions present during life. We believe that the presence of the colon bacillus at autopsies is occasionally due to agonal invasion of the body and usually due to post-mortem outgrowth through the body from the intestine.

In addition to the use of blood cultures, certain evidence has been obtained by the author (60) from experiments with blood serum which I will briefly refer to here. It was thought that an explanation of general bacterial invasion of the body in chronic disease could be found in a loss or weakening of the germicidal properties of the blood, but the balance of evidence of previous work which was confirmed by a series of experiments by the author indicates that the blood serum, even in healthy individuals, is not appreciably germicidal for the pus organisms, which disposes of this theory so far as the pus organisms are concerned. On the other hand, it is well known that normal human serum is germicidal to the colon bacillus, and the author has found that the serum, as a rule, retained this property for this organism in cases of severe chronic disease up to the end of life, and in about half the cases examined, even for several hours after death. In only two cases in the series was there any evidence of loss of germicidal power before death. Now in this fact that the serum retained its germicidal properties in most cases until death and in many cases after it, we have a strong additional reason why agonal invasion of the blood by intestinal germs is not likely to occur in the majority of cases, even of severe chronic disease.

CONCLUSIONS.

Our conclusions from the literature and our own experiments may be summarized as follows:

I. Blood for bacteriological examination during life should be taken directly from the veins and in considerable quantity.

II. Resorption of toxines is the most important feature in cases

of sepsis; pyogenic bacteria invade the general circulation in a rather small proportion even of severe cases, and, as a rule, late in the course of the disease.

III. A general infection by the pneumococcus can be demonstrated occasionally in the late stages of acute lobar pneumonia.

IV. The value of blood cultures as a means of diagnosis in obscure cases of sepsis is limited by the fact that invasion of the blood by the specific organism cannot be demonstrated during life in the majority of cases. Positive cultures are very valuable; negative cultures do not exclude local septic infections.

V. The detection of specific bacteria in the blood of cases of sepsis and of pneumonia gives a very unfavorable prognosis in most cases.

VI. General terminal infections with pyogenic cocci occasionally occur as an immediate cause of death in chronic disease. Local infectious processes play this part more frequently.

VII. As far as our experiments have shown, invasion of the blood by bacteria during the death agony, with subsequent distribution of the germs to the organs by the circulation, is a rather uncommon occurrence.

VIII. Owing to the relative infrequency of agonal invasion, we believe that in the majority of cases where the autopsy is performed promptly after death, the bacteria which are found in the organs succeeded in reaching these organs previously to the death agony, and are associated with the course of the disease.

IX. The presence of bacteria in the organs of late autopsies is due in many cases to post-mortem extension from one organ to another, and in some cases to the post-mortem growth of small numbers of germs which were distributed to the organs by means of the circulation.

In closing I wish to express my thanks to Dr. J. H. Wright, Director of the Pathological Laboratory at the Massachusetts General Hospital, for his kind assistance in my work.

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A CASE OF SUSPECTED RABIES WITH ISOLATION OF BACILLUS DIPHTHERIÆ FROM THE CENTRAL NERVOUS SYSTEM.

By GEORGE DOUGLAS HEAD, M. D., AND LOUIS BLANCHARD WILSON, M. D.

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CLINICAL REPORT.

By George Douglas Head, M. D.

On the morning of September 28, 1897, Mrs. L. R. presented herself for the surgical treatment of a bite of the left cheek. She stated that about 4 o'clock that morning she was awakened by a noise in the chicken coop, and hurrying on her clothing went out to investigate. As she pushed open the door of the coop an animal sprang out upon her, biting her on the left cheek. The wound bled profusely and to stop the hæmorrhage she applied some liniment and a bandage.

When she presented herself for treatment, five hours after the accident, there were two sharp, deep punctures on the left cheek, one-half an inch apart, sinking deeply into the underlying tissue, from which on pressure a bloody grumous fluid exuded. The wound was scrubbed with soap and water, washed out with peroxide of hydrogen and packed with gauze. Two days following, the same treatment was repeated. At the third dressing, as the wound seemed clean, it was allowed to granulate over and the patient was dismissed.

Nothing more was heard of the patient until December 3, two months and five days following the bite. On that day the patient again came complaining of severe pain and numbness over the left cheek in the region of the previous bite. The office notes were as follows:

Woman, 32 years, well nourished, married, two children, one miscarriage, mother and father and one sister living; mother well; father has ill health, of which the cause was not ascertained, previous history uneventful. Had neuralgia of right side of face some years ago. No other ailments. No history of hysteria or other neuroses.

The pain of which the patient complains is localized on the left cheek in the region of the scar of the previous bite. It commenced

two days ago as a mild aching and numbness in that region and has been gradually growing more severe. There is little swelling and only slight tenderness on pressure over the area of pain. There is complaint of some difficulty in opening the mouth owing to pain in the temporo-maxillary articulation. Patient complains of loss of sleep. Temperature 98.8° F. Pulse 100.

Diagnosis: facial neuralgia. Treatment: phenacetine and salicylates.

Dec. 4. Patient at office. Tenderness most marked over the site of the bite. Pain paroxysmal in character, running along the ramus of lower jaw, up the side of head, in front and back of left ear, and back along the left sternocleidomastoid muscle. As patient complains of difficulty in swallowing I give her a glass of water to drink. She raises the glass to her lips, gives a short, jerky inspiration, and swallows the liquid. Suspecting rabies, I call Dr. Hunter in consultation.

Patient carefully examined. No throat lesions. No decayed teeth. No ear trouble. Tenderness sharply localized over the area supplied by middle branch of fifth nerve. Temp. 99.4°. Diagnosis: neuralgia of middle branch of fifth nerve or beginning rabies. Treatment, morphine.

December 5. Fifth day of disease. Called at 4 a. m. Patient has spent a bad night with little sleep. Excruciating pain over same area as described. Mouth opened with more difficulty. Can swallow saliva, but if she attempts to drink water, tea or milk she takes a number of short, quick, catchy inspirations, her eyes start from her head, she looks wildly about, then swallows the fluid with a gulp. The swallowing seems a great effort and tires her. Temperature 99°. Pulse 108. Heart, lungs, and abdomen normal. No enlarged spleen. Bowels constipated. Urine, acid, 1024. No albumin, or sugar. Urea 2.5 per cent. No casts, blood or leucocytes. Diagnosis: suspected rabies.

December 5, 4 p. m. Patient resting easier. Laryngeal and respiratory spasms more pronounced. Now even the taking of a glass of fluid into her hand causes a faint spasm. As she puts the liquid to her lips her hand will shake violently, the catchy inspirations will begin, and it seems almost impossible for her to swallow. She can swallow solids, though with some difficulty. With the exception of the laryngeal spasms and the pain, she seems perfectly normal. Sits up in a chair, talks with her friends, and is undisturbed mentally. Temperature 99.2°. Pulse 110, irregular and rapid. Respirations normal.

December 6, 8 a. m. Drs. Hunter and Sweeney in consultation. Sleepless night. Has taken little nourishment and no fluids. Complains much of thirst. Bowels moved. Temperature 100°. Pulse 110.

Respiratory and laryngeal spasms on taking fluids. Applications of snow to face or arm causes the spasms. Pupils react to light and accommodation. Reflexes feebly present. Knee-jerk absent in right leg, present in left leg. No ankle-clonus. No areas of hyperæsthesia. On left tonsil white exudate which is removed with swab, supposed to be due to a strong solution of sulphuric acid used by her mother for swabbing out the throat. Heart, lungs, and abdomen normal. Treatment: morphine; fluid enemata.

December 6, 4 p. m. No change. Slept some after the morphia. Laryngeal and respiratory spasms present on taking liquids; also, in less pronounced degree, on taking solids. Complains bitterly of thirst but can scarcely be induced to attempt to drink. Temperature 101°. Pulse 108. Respiration normal. Cocainized pharynx and gave one pint of milk by stomach-tube.

December 6, 12 p. m. Pain in face not complained of. Laryngeal and respiratory spasms manifested as before. Temperature 101°. Pulse 110. Gave one pint of milk by stomach-tube: also morphia.

December 7, 9 a. m. Dr. Jones in consultation. Patient seems brighter and more cheerful. Slept some during the night. Can swallow oysters and crackers moistened in water. On offering her some milk to drink she pleads not to be asked to try but finally takes a swallow only to exhibit the same laryngeal and respiratory spasms in a more pronounced degree. Now as she attempts to take fluid her eyes bulge, she clutches a support to sustain herself, her whole body seems in a violent tremble, and as the fluid touches her lips there comes a series of quick respirations, followed by a convulsive cough which blows the milk out of her mouth over herself and the bed clothes. Temperature 100.5°. Pulse 120. Bowels moved. Gave one pint of milk by stomach-tube; also bromides.

December 7, 5 p. m. Temperature 101.5°. Pulse 120. Tongue coated white, pharynx normal. Eyes bright. Mind clear. No sleep. Treatment, morphia. Milk and water by stomach-tube.

December 7, 12 p. m. Patient seems languid and tired. Wants to sleep but cannot. Face flushed, eyes bright. Laryngeal and respiratory spasms easily produced. Temperature 102°. Pulse 130. No rose spots. No tympanites. No enlarged spleen. Heart and lungs normal. Give eggs and one pint of milk by stomach-tube, also morphia to induce sleep.

December 8, 8 a. m. Dr. Hunter in consultation. Patient passed a good night. Laryngeal and respiratory spasms present. Knee-jerk

absent. Pupils react to light and accommodation. No ankle-clonus. Superficial reflexes normal. No rose spots. No enlarged spleen, nor tympanites. Temperature 103.2°. Pulse 130. Take blood for Widal test. Give 2 eggs, milk and whiskey by stomach-tube. No morphia.

December 8, 4 p. m. Patient complains of pain in upper abdomen, extending around the body. Stomach distended with gas. Temperature 102.8°. Pulse 120. Blood examination: leucocytes, 6000; red corpuscles, 5,500,000. Hæmoglobin 90 per cent. Widal test (State Board of Health Laboratory) gives a positive typhoid reaction (see p. 456). Give 2 eggs, milk and whiskey by stomach-tube.

December 8, 8 p. m. Patient seems better. Complains of pain in limbs and back. Mind clear. Laryngeal and respiratory spasms present. Temperature 102.5°. Pulse 120. Tongue coated white. No morphia.

December 9, 8 a. m. Patient complains of pain in abdomen which is relieved when bowels move. Laryngeal and respiratory spasms still severe. Lungs and heart normal. No rose spots. No tympanites. No enlarged spleen. Urine 1024, acid, a trace of albumin, no sugar, urea 3 per cent. No casts, leucocytes or blood corpuscles. Temperature 102.2°. Pulse 120. Ate six oysters and took a few swallows of milk.

December 9, 8 p. m. Patient slept some during the day. Laryngeal and respiratory spasms not so pronounced. Temperature 103.2°. Pulse 120. Knee-jerk absent. No ankle-clonus. Sensation normal. Grasp of right and left hand unimpaired. Pupils react to light and accommodation. Patient drinks a cup of milk. No morphia.

December 10, 8 a. m. Patient delirious with rational intervals. No sleep during the night. Had hallucinations of being drowned, of the house on fire, of persecution. Occasionally patient had a general tremor of the whole body. Temperature 102°. Pulse 110, irregular. Had eaten some bread and milk.

December 10, 8 p. m. Patient extremely nervous. Fingers twitch. Casts furtive glances this way and that. Low, incoherent muttering. Temperature 101°. Pulse 130. Passes urine, and bowels move. Has taken milk and broth.

December 11, 3 a. m. Called in haste. Patient in violent delirium. Had gotten out of bed, knocked down nurse, and run out of doors. Patient tries to tear her clothing, bites at those about her. Has to be held by force. Quieted by one-half grain of morphia. Temperature 103°. Pulse 120. Urine, 1022, large amount of albumin. No sugar. Urea 4 per cent., a few pus cells, with a few hyaline casts and cylindroids.

December 11, 8 a. m. Dr. Hunter in consultation. Patient perfectly rational. Recognizes those about her. Talks sensibly, but there are occasional twitchings about the eyes. Quick glances this way and that. Takes little nourishment. Temperature 102.2°. Pulse 110. Calomel administered to move the bowels.

December 11, 8 p. m. Patient seems worse. Muttering delirium. Constant twitching of muscles of whole body. Eyes bright, face red and injected. Tongue flabby with pasty coat. Passes urine and bowels move. Temperature 104°. Pulse 110. Treatment: morphia ($\frac{1}{2}$ gr.), whiskey and strychnia.

December 12, 10 a. m. Patient does not recognize me. Seems more quiet, inclined to lie still. Has taken three or four cups of water and some milk. She drinks now without the occurrence of spasms. Severe attacks of trembling of whole body or of one limb, then of another. Urine 1028. Large amount of albumin, a few hyaline casts. Temperature 102.2°. Pulse 140. Treatment: whiskey and strychnia.

December 12, 8 p. m. Dr. Sweeney in consultation. Patient worse. Has hoarse cough, rapid respirations. There is an increased flow of saliva from mouth. Muttering delirium; picking at bedclothes; violent shaking of limbs. Heart, normal. Lungs: fine, mucous rales at bases. Abdomen normal. Take blood for Widal test.

December 13, 8 p. m. Thirteenth day of disease. Patient has had no sleep, speech thick and incoherent. Can be aroused but if left alone lies in a semi-conscious state. Temperature 102°. Pulse 130. Blood examination: red corpuscles, 5,120,000; leucocytes, 14,500.

Widal test (State Board of Health Laboratory) gives a positive typhoid reaction (p. 457). Notwithstanding this report there is little clinical evidence of typhoid fever. The leucocyte count in the second week of uncomplicated typhoid would not be increased. Typhoid fever is therefore not the probable diagnosis.

The laryngeal and respiratory spasms are again produced by the swallowing of solids or liquids or the putting of one's hands on the body or wiping away mucus from the mouth.

December 13, 8 p. m. Patient is unconscious. Has taken no nourishment. Short, sharp, muscular contractions sweep over the arms and legs. Temperature 102°. Pulse 140. Respirations feeble and superficial. There is an occasional mucous rattle in the trachea. It does not seem possible that the patient can long survive.

December 14. Patient died at 5 a. m. in an unconscious state, the respirations being previously rapid and superficial and the heart's action irregular.

Autopsy, 16 hours after death, the body having been kept at low temperature. A complete examination was not permitted.

Body well nourished. Rigor mortis pronounced. Scar on left cheek. Patchy livores mortis over posterior surface of body.

Dura adherent along superior longitudinal sinus. No other adhesions. Slight hyperæmia of dura, which is not thickened. Pia somewhat hyperæmic, free from exudate.

An incision was made with sterilized scalpels into left lateral ventricle and 1 cc. of clear serum was removed in a sterilized pipette, the end of the pipette being immediately sealed in a flame.

Cerebral convolutions well developed and normal. No exudate or effusion. No adhesions. No areas of softening. Vessels somewhat injected. Punctate oozing on section of brain. Meningeal vessels normal. No tubercles, emboli, thrombi, or adhesions. Cerebellum normal. Basal ganglia normal.

The third ventricle was opened with a sterile knife, and by means of a sterile pipette some bloody fluid was withdrawn, the ends of the pipette being at once sealed in a flame.

The medulla and pons Varolii were removed with sterilized instruments and placed in a sterilized flask.

A complete post-mortem examination not being allowed, the abdominal cavity was opened by a median incision only 10 cm. long. There were no adhesions or increase of fluid in the peritoneal cavity. The peritoneum and the intestines, both small and large, showed no abnormality. Parts of the cæcum and ileum were removed. Peyer's patches and the solitary follicles were not affected.

The right kidney was somewhat enlarged and congested. Capsule stripped off with ease. Dark red areas were scattered over the cortex. The left kidney presented the same appearance. Pieces of kidney removed and placed in 95 per cent alcohol. Liver appeared normal. Spleen was not enlarged. All tissues and fluids taken at the autopsy were kept on ice until taken to the State Board of Health Laboratory 13 hours later.

PATHOLOGICAL AND BACTERIOLOGICAL REPORT.

By Louis Blanchard Wilson, M. D.

(From the Bacteriological Laboratory of the Minnesota State Board of Health.)

The connection of the laboratory with the case of which the clinical history has been given by Dr. Head, began with the receipt, on Dec. 8, 1897, of a specimen of blood for examination for the serum reaction for typhoid fever. The specimen was dried on a visiting card, and con-

sequently only a colorimetric estimate of the dilution employed could be made. The reaction was present in a dilution of 1:25 so estimated. On Dec. 13, another specimen collected, weighed and accurately diluted after the author's method,* was found to give a good reaction in a dilution of 1:25, partial in 1:50, and a trace in 1:100.† In consequence of these two reactions special care was taken in the bacteriological examination of the specimens secured at the autopsy.

On December 15, at 9 a. m., there were received at the laboratory: one sealed bulb, containing about 1 cc. of fluid from the left lateral ventricle of the brain; a similar bulb, with about 0.5 cc. of fluid from the third ventricle; the lower two-thirds of the medulla, with about 1 cm. of the cervical cord, in a sterile flask plugged with cotton wool and about 2 ccm. of the pons, which had been placed in 4 per cent formaldehyde solution at the autopsy; and a portion of one kidney wrapped in gauze. Later there was also received another portion of the kidney, which had been placed in 80 per cent alcohol at the time of the autopsy.

Coverslip preparations. Four coverslip preparations each were made from the fluid from the left ventricle, from that from the third ventricle, from the substance of the medulla and from that of the kidney. They were stained with methylene-blue and eosine. Those from the lateral ventricle showed very few cocci, and two or three bacilli rather unevenly stained. In those from the medulla were found a few isolated cocci. No bacteria were found in any of the others.

Sections.—Kidney. In a portion of kidney placed in alcohol at the autopsy, many of the glomeruli were apparently normal. A few showed more or less complete hyaline degeneration and some cloudy swelling, with disappearance of the nuclei of the epithelium of the convoluted tubules. No bacteria could with certainty be distinguished.

Nerve tissues. Portions of tissue from the pons, medulla and cord were fixed and hardened in 96 per cent alcohol, embedded in celloidin and in paraffine, and sections 3, 5, 10 and 20 microns in thickness cut therefrom. These were stained by Nissl's method, by Weigert-Gram,

* Wesbrook and Wilson, *Philadelphia Med. Journ.*, 1898, i, 549.

† These reactions are perhaps explicable on either of two hypotheses: (*a*) a typhoid infection existed, and other pathological evidence of it was not obtained, owing to the incomplete autopsy permitted; (*b*) though no history of a previous typhoid fever was elicited, the exclusion of a previous typhoid infection would seem to be almost impossible, since the patient had been for years a resident of a city in which for a decade typhoid fever has been endemic, and from the public water-supply of which the typhoid bacillus was isolated a year ago (See Wilson and Wesbrook, *British Med. Journ.*, 1897, ii, 1774.)

and by Löffler's methylene-blue alone. The walls of some of the vessels were thickened. Where this occurred, the vessels were filled with blood, and the walls infiltrated with leucocytes. In some instances the perivascular spaces were completely filled with leucocytes. No accurate determination of the condition of the nerve fibres could be made by the methods employed. With Nissl's stain, many of the cells of the grey matter of each of the regions studied appeared to be perfectly normal. A large number of these cells, however, showed chromatolysis in varying degrees, from simple irregularity and subdivision of the Nissl granules to the almost complete disappearance of the chromatin. In most instances the periphery of the cell-body was least affected. Where chromatolysis was at all marked, the nucleus and nucleolus were in most cases eccentrically placed. In a few instances, the nucleus was distorted in shape; in others enlarged, irregularly crescentic nucleoli were present. Apparent complete absence of processes from some of the most affected cells was noted.

The foregoing changes were common to pons, medulla and cord. In addition, in the medulla and cord—which had been 13 hours longer out of preserving fluid—were found occasional cells, in which the nucleoli, while not enlarged, were partially divided, the nuclei stained light blue throughout and the cell-body was filled with well-stained, finely divided granules, the otherwise even distribution of which was broken up by "vacuoles." The writer, however, agrees with Ewing * and others in considering these cadaveric rather than vital changes.

In the sections of the pons and medulla stained by Weigert-Gram and by Löffler's methylene-blue, several groups of bacilli were found. These in size, shape and staining characteristics could not be distinguished from *Bacillus diphtheriae,* and, in view of the cultural findings to be described later, in all probability were such. The groups consisted of two to five individuals each and were located within the bodies of the larger nerve cells. They were not observed in the vessels or perivascular spaces.

Though many observers, among others Löffler,† Kolisko and Paltauf,‡ Strelitz,§ Johnston,|| Abbott and Ghriskey,¶ Flexner,** Frosch,††

* Ewing, J., *N. Y. Med. Record*, 1898, liii, 513.

† Löffler, *Mittheil. a. d. k. Gesundheitsamte*, 1884, ii, 421.

‡ Kolisko and Paltauf, *Wien. kl. Wochenschr.*, 1889, ii, 147.

§ Strelitz, *Arch. f. Kinderheilk.*, 1891, xiii, 468.

|| Johnston, *Montreal Med. Journ.*, 1891, xx, 161.

¶ Abbott and Ghriskey, *Bulletin of the Johns Hopkins Hospital*, 1893, iv, 29.

** Flexner, Ibid, iv, 32.

†† Frosch, *Zeitschr. f. Hyg.*, 1893, xiii, 49.

Booker,* Canon,† Kutscher,‡ Wright§ and Stokes,|| Belfanti,¶ Kanthack and Stephens,** and Flexner and Anderson,†† (the last two papers contain good reviews of the previous literature) have demonstrated the presence of B. diphtheriæ in the internal organs of patients dead of clinical diphtheria, or similarly in animals dead from experimental inoculation, yet, so far as I am aware, Frosch alone has hitherto shown their presence within the central nervous system.

Cultures. Tubes of plain bouillon, glycerine agar and glycerine serum were sown direct from the bulb of ventricular fluid, from the substance of the central portion of the medulla, and from the kidney. The latter was then wrapped in 1:1000 bichloride gauze, incubated for 24 hours and second cultures were made.

Of four tubes of solid media inoculated from the right ventricle one —serum—developed 3 colonies of a bacillus indistinguishable in cover-slip preparations from B. diphtheriæ—and shown later to be such—and one colony of a diplococcus. Another—agar—developed one colony of a diplococcus. The broth cultures gave both B. diphtheriæ and diplococci. Four tubes from the third ventricle gave two to ten colonies each (broth abundant growth), about two-thirds of which were B. diphtheriæ and the remainder streptococci and diplococci.

Though the sowings from the medullary substance were abundant only two of seven tubes showed growth. One of these—agar—gave one colony of diplococci and the other—broth—many B. diphtheriæ and diplococci.

From the kidney a few colonies of a large white staphylococcus and an abundant growth of B. coli communis developed.

The diphtheria-like bacillus was isolated and carefully compared with specimens of known virulent diphtheria bacilli. Parallel cultures were made on Löffler's and glycerine serum, on plain glycerine and litmus-lactose agar, on plain and glucose gelatine, on potato, in plain glycerine, glucose and sugar-free bouillon, and in plain and litmus milk. The cultures were compared as to rate and character of growth, reaction of the media and appearance of stained preparations. In no particular did the organism under investigation differ materially from those with

* Booker, *Arch. of Pediatrics*, 1893, x, 642.

† Canon, *Deutsche med. Wochenschr.*, 1893, p. 1038.

‡ Kutscher, *Zeitschr. f. Hyg.*, 1894, xviii, 167.

§ Wright, *Boston Med. and Surg. Journ.*, 1894, cxxxi, 329; 357.

|| Wright and Stokes, Ibid, 1895, cxxxii, 271; 293; 330. Stokes, ibid, 1895, cxxxiii, 581.

¶ Belfanti, *Lo Sperimentale, Sez. biol.*, 1895, xlix, 278.

** Kanthack and Stephens, *Journ. of Path. ana Bact.*, 1896-7, iv, 45.

†† Flexner and Anderson, *Bulletin of the Johns Hopkins Hospital*, 1898, ix, 72.

which it was compared. In the earlier cultures—on glycerine serum—it produced involution forms (large clubbed and "ghost" forms) somewhat more quickly than the controls, but this property was lost by the time the tenth generation was reached.

Animal inoculations with cultures. In order to determine the virulence and identity of the bacillus, guinea-pigs were inoculated, with the results shown in Table I.

It will be seen from the experiments recorded in Table I that the bacillus under investigation possessed an ordinary degree of virulence, that the autopsy findings were the same as those in death from known diphtheria bacilli, that in every instance the organism was recovered in pure culture and unchanged morphologically or culturally from the seat of inoculation (though from no other part of the body) and that diphtheria antitoxine invariably protected against otherwise fatal doses of the organism.

For experimental purposes a toxine was prepared by growing the bacillus—tenth generation from the original source—for 23 days in sugar-free bouillon in the incubator, and filtering through a Chamberland filter. Its toxicity was then tested with the results shown in Table II, p. 462.

Rabbit No. 1, weight 2860 grammes, whose serum had been shown by inoculation tests to be not antidiphtheritic, was then immunized by increasing doses of the toxine prepared as described above, and finally by inoculation with a living culture of the bacillus. The rabbit was then bled and its serum used as noted in the experiments given in Table III, p. 463.

Inoculations to determine the presence of the virus of rabies. About 0.5 ccm. from the central portion of the medulla of Mrs. R., received as noted above, was removed in a sterile manner and with sterile 0.67 per cent sodium chloride solution made into as thick an emulsion as could be passed through an ordinary hypodermic needle. With this emulsion the following inoculations were made:

Rabbit No. 2. Weight 1120 grammes. Inoculated December 15, 1897, 11 a. m., in left subdural space with 0.2 ccm. of emulsion above mentioned.

Appeared perfectly well until 9 a. m. Jan. 4, 1898, 20 days after inoculation, at which time it showed disinclination to move, and when made to hop exhibited weakness in posterior extremities with slight incoördination. Temperature 35.7° C. 11.30 to 12 m. incoördination became much more marked. Animal very restless, moving about constantly and grinding its teeth frequently. During afternoon animal

GUINEA-PIG INOCULATIONS WITH BACILLUS X.

No.	Weight.	Subcutaneous inoculation in right groin.	Protected (?) by subcutaneous injection in left groin of	Period from inoculation till death.	Positive gross findings at autopsy.	Glycerine serum and bouillon cultures made at the autopsy; examined after 21 and 48 hours in the incubator.
	Grms.					
1	520	0.5 cc. 40-hr. plain bouillon culture of Bacillus X.	Not protected.	4 days.	Intense œdema at seat of inoculation. Liver slightly enlarged. Meninges slightly congested.	Seat of inoculation = Bacillus X, pure. Heart's blood = sterile. Spleen = sterile. Ventricles and substance of brain and spinal cord = sterile.
2	450	Same dose and culture as with No. 1.	Not protected.	4 days.	Intense œdema at seat of inoculation. General œdema over whole ventral portion of body.	Same as in No. 1.
3	390	"	60 units, N. Y. City B'd of Health diphtheria antitoxine.	40 days.	Small area of extravasation at seat of inoculation. Animal much emaciated.	All cultures sterile.
4	330	"	30 units of the same.	32 days.	Animal much emaciated.	All cultures sterile.
5	430	0.43 cc. 40-hr. sugar-free bouillon culture of Bacillus X.	Not protected.	2 days.	Intense congestion and œdema at seat of inoculation. Spleen enlarged, congested.	Same as in No. 1.
6	410	0.41 cc. of same culture as in No. 5.	Not protected.	2½ days.	Same as No. 5, and, in addition, kidneys much congested.	Same as in No. 1.
7	250	0.25 cc. of same culture as in No. 5.	Not protected.	2 days.	Same as No. 5, and, in addition, liver much congested.	Same as in No. 1.
8	410	0.41 cc. of same culture as in No. 5.	50 units, Parke, Davis & Co.'s antidiphtheritic serum. (Mixed with bacteria immediately before their injection into right groin.)	38 days.	Area of slight infiltration at seat of inoculation. Three abscesses in liver. Subcapsular abscess on surface of right kidney. Spleen pale.	Seat of inoculation, heart's blood, spleen and brain substance = sterile. Liver abscess, Kidney abscess, } Short, thick bacilli, not diphtheria.
9	160	0.16 cc. 70-hr. plain bouillon culture of Bacillus X.	Not protected.	2 days.	Much œdema at seat of inoculation. Kidneys pale. Spleen much congested.	Same as in No. 1.
10	600	0.6 cc. of same culture as in No. 9.	Not protected.	3 days.	Same as in No. 9, and, in addition, adrenals and liver congested.	Same as in No. 1.
11	275	0.275 cc. of same culture as in No. 9.	150 units, P., D. & Co's antidiphtheritic serum.	14 days.*	Lungs fill thoracic cavity, in state of congestion. Kidneys slightly softened.	Heart's blood = staphylococci. Seat of inoculation, spleen and brain substance = sterile.
12	250	0.5 cc. of same culture as in No. 9.	150 units of same.	18 days.*	As in No. 11.	As in No. 11

* These 2 guinea-pigs were from a lot of 50 received but a few days before, and all the others of which died of pneumonia (?) in periods of from 1 to 3 weeks after their receipt in the laboratory.

TABLE II.

GUINEA-PIG INOCULATIONS WITH TOXINE FROM BACILLUS X.

No.	Weight.	Subcutaneous inoculation in right groin.	Protected (?) by	Period from inoculation till death.	Positive gross findings at autopsy.	Glycerine serum and bouillon cultures made at autopsy and examined after 24 and 48 hours in the incubator.
13	300	0.15 cc. filtered toxine — prepared by growing Bacillus X 23 days in the incubator in sugar-free bouillon.	Not protected.	3½ days.	Much œdema at seat of inoculation. Spleen enlarged and congested. Liver enlarged and congested.	Seat of inoculation, Spleen, Heart's blood, Ventricles and substance of brain and spinal cord, = sterile.
14	310	0.31 cc. of same material as in No. 13.	Not protected.	1½ days.	Same as in No. 13.	Same as in No. 13.
15	330	0.15 cc. of same material as in No. 13.	0.3 cc. serum of Rabbit No. 1. (Normal —afterwards immunized with toxine noted in G. P. 13.) Serum mixed with toxine immediately before inoculation.	3½ days.	Same as in No. 13.	Same as in No. 13.

TABLE III.

GUINEA-PIGS PROTECTED WITH ANTITOXIC SERUM PREPARED WITH BACILLUS X.

No.	Weight.	Subcutaneous inoculation in right groin.	Protected (?) by subcutaneous injection of	Period from inoculation till death.	Positive gross findings at autopsy.	Glycerine serum and bouillon cultures made at the autopsy; examined after 21 and 48 hours in the incubator.
	Grms.					
16	400	0.25 cc. 31-day sugar-free broth culture of known B. diphtheriæ (series De C).	Not protected.	$1\frac{1}{2}$ days.	Intense œdema and congestion at seat of inoculation. Spleen congested.	Seat of inoculation = B. diphtheriæ, pure. Heart's blood, Spleen, Ventricles and substance of brain and cord, } = sterile.
17	370	Same dose and culture as with No. 16.	0.5 cc. serum from Rabbit No. 1. (Immunized with toxine from Bacillus X.) Mixed with bacteria immediately before inoculation.	36 days.	Slight area infiltrated at seat of inoculation. Entire lungs in red hepatization.	Seat of inoculation = sterile. Heart's blood = staphylococci. Spleen = staphylococci. Brain and cord = sterile.
18	810	0.81 cc. 70-hr. plain bouillon culture of B. diphtheriæ (series De C.).	Not protected.	$1\frac{1}{2}$ days.	Considerable œdema and congestion at seat of inoculation. Kidneys congested. Liver and spleen pale.	Same as in No. 16.
19	140	0.28 cc. same culture as with No. 18.	0.3 cc. same serum as with No. 17.	26 days.	Right lung œdematous.	Same as in No. 17, except spleen sterile.
20	150	0.15 cc. 70-hr. plain bouillon culture of known B. diphtheriæ (series W. W.).	Not protected.	$2\frac{1}{2}$ days.	Intense congestion and œdema at seat of inoculation. Kidneys and adrenals congested. Spleen enlarged. Liver pale, mottled.	Same as in No. 16.
21	115	0.12 cc. same culture as with No. 20.	0.5 cc. same serum as with No. 17.	Still alive after 96 days		

remained quiet. Jan. 6, 1898, 9 a. m., rabbit found lying on side, stretched out, head thrown backwards, all power of motion in posterior extremities apparently gone. Temperature 35.5° C. 1 p. m., condition unchanged. Sniffed at food which was placed near nose but did not attempt to eat. (Culture from throat taken at 5 p. m., Jan. 4, showed staphylococci, streptococci and short slender bacilli, unidentified.) Jan. 6, 8 p. m., 22 days after inoculation, rabbit was found dead and in rigor mortis.

Autopsy 9 p. m., Jan. 6. Marked injection of vessels of pia; minute extradural plastic exudate at seat of inoculation; mouth and trachea clear of mucus; vessels of trachea not congested; small light-colored patch on inferior border of median lobe of liver; bladder much distended and its vessels injected; other organs normal.

Cultures made from pia, fourth ventricle, and heart's blood, all remained sterile. Those from substance of medulla gave abundant pure growth of a bacillus with the same morphological and cultural characteristics as that obtained from the original source and identified as the diphtheria bacillus.

Rabbit No. 3. Weight 1270 grammes. Inoculated at same time (Dec. 15), in the same manner, and with the same substance and dose as Rabbit No. 2.

Jan. 5, 9 p. m., 21 days after inoculation, animal showed first symptoms. History similar to that of Rabbit No. 2, except that at no time was any excitement present, and that much flowing of saliva was noted throughout the second day after the appearance of symptoms. Death Jan. 6 at 11 p. m., 22 days after inoculation.

Autopsy findings parallel with those of No. 2, except that the throat was filled with mucus and the trachea was slightly injected in upper portion. The liver was apparently normal. Cultures from surface of brain at seat of inoculation and heart's blood remained sterile. Those from substance of medulla developed pure growth of B. diphtheriæ.

Though the Bacillus diphtheriæ was recovered in pure culture from the central nervous system of Rabbits Nos. 2 and 3, yet the clinical history and gross autopsy findings in the animals were so like those observed in rabbits inoculated subdurally with the virus of known rabies that it was impossible to decide whether death had been caused by the virus of rabies, by the diphtheria bacillus, or by a combined infection with the two. Hence the following inoculations were made from the substance of the medulla of Rabbit No. 2.

Rabbit No. 4. Weight 1390 grammes. Inoculated 2 p. m., Jan. 6, 1898, in left subdural space with 0.2 ccm. of thick emulsion of medulla

of Rabbit No. 2 in 0.67 per cent. NaCl. solution. (A small quantity of the emulsion escaped from the wound in the dura on the withdrawal of the needle.)

Rabbit remained apparently well up to and including 1 p. m., Feb. 1. 1898. Found dead and in rigor mortis 9 a. m., Feb. 2, 27 days after inoculation.

At *autopsy* animal very much emaciated. Pia slightly congested. Trachea normal. Liver congested, not markedly enlarged. Gall bladder much distended. Urinary bladder distended. All other gross findings negative. Cultures from surface of pia, heart's blood and gall bladder all remained sterile after 7 days in the incubator. Those from the substance of the medulla developed a few colonies of B. diphtheriæ.

Rabbit No. 5. Weight 1030 grammes. Inoculated Jan. 6, 2 p. m., in left subdural space with 0.2 ccm. of the same emulsion as that used in inoculating Rabbit No. 4 (*i. e.* medulla in physiological salt solution).

Jan. 23, 10 a. m., 17 days after inoculation, the rabbit showed disinclination to move; when compelled to hop exhibited slight posterior incoördination. Temperature 38°.5, 5 p. m. Symptoms grew rapidly worse during afternoon—animal lying on side with posterior limbs completely paralyzed. Found dead Jan. 24 at 9 a. m., 18 days after inoculation.

Autopsy. Body not emaciated. Meninges very intensely congested. Trachea slightly congested. Heart and adjacent vessels engorged with blood. Liver very dark, congested, enlarged and friable. Spleen pale. Right kidney slightly congested. Other gross findings negative.

Serum and broth cultures from the meninges and substance of medulla gave a few colonies of B. diphtheriæ. Similar cultures from the ventricles of the brain, the heart's blood, and the spleen, remained sterile after 5 days in the incubator.

Rabbit No. 6. Weight 1210 grammes. Inoculated Jan. 6, 1898, 2 p. m., in left subdural space with 0.2 ccm. of emulsion prepared by rubbing up an unmeasured portion of the medulla of Rabbit No. 2 with P., D. & Co.'s diphtheria antitoxine (date of Oct. 9. 1897. 2000 units to bulb). The emulsion was as thick as could be passed through the needle of a hypodermic syringe.

This rabbit has shown no symptoms up to the present time, 130 days after the inoculation.

Rabbit No. 7. Weight 970 grammes. Was inoculated Jan. 6. 1898. in the left subdural space with 0.25 cc. of the same emulsion as that used in inoculating Rabbits Nos. 4 and 5. Immediately preceding this inoculation the animal had been given subcutaneously in the right

groin 1 cc. (about 500 units) of antidiphtheritic serum (P., D. & Co.'s issue of Oct. 9, 1897). This rabbit has exhibited no symptoms up to the present time, 130 days after the inoculation.

The extreme difficulty of procuring rabbits at the time of death of Nos. 4 and 5, coupled with the strong belief that Nos. 6 and 7 would eventually die of rabies, was deemed sufficient reason for not carrying forward the series from the medullæ of those already dead.

Effects of association of the virus of rabies with diphtheria antitoxine and B. diphtheriae.—The experiments on Rabbits Nos. 6 and 7 indicate that the virus of rabies was absent from the material used to inoculate these animals and presumably also from the medulla of the patient, but before this inference could be justifiably drawn it was necessary first to determine what influence diphtheria antitoxine may have upon the virus of rabies or upon a combination of this virus with the diphtheria bacillus.

In numerous experiments made in this laboratory large doses of antidiphtheritic serum have been administered subcutaneously to rabbits just prior to their inoculation with the fixed virus of rabies, and in other instances with the virus of street rabies, without any appreciable effect either on the time of onset or the character of the symptoms. Equally without effect upon the manifestations of rabies was the mixture of the rabic virus with diphtheria antitoxine before inoculation of the animal.

The experiments recorded in Table IV (p. 467) were made to determine the effect, if any, on the incubation period, symptoms, etc., of inoculation of rabic virus simultaneously with diphtheria antitoxine and diphtheria bacilli.

It will be observed from Table IV that Rabbits Nos. P85, P87, 268, 270, were used merely as controls, the first two being inoculated in the ordinary manner with the fixed virus of rabies from two different animals, and the last two with the virus of street rabies, second generation from a rabid dog. In Nos. P86 and P88 the addition of diphtheria antitoxine and virulent *B. diphtheriae* to the fixed virus some time before inoculation did not appear to alter in any way either the incubation period, the train of symptoms, or the autopsy findings. Rabbits Nos. P109, P111, P113, P115, P117 and P121 of Table V may also be cited in this series. The diphtheria bacilli had been in contact with the living nerve tissue and rabic virus for from 2 to 15 days in the material with which these latter animals were inoculated, yet the rabic virus appeared unchanged thereby.

In Rabbits Nos. 269 and 271, in the inoculation of which to the rabic

TABLE IV.

INOCULATION OF RABBITS WITH RABIC VIRUS COMBINED WITH DIPHTHERIA BACILLI AND DIPHTHERIA ANTITOXINE.

No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days to onset of symptoms.	Symptoms.	No. of days from inoculation till death.	Autopsy.
P. 85 (control)	1460	In left subdural space with 0.2 cc. of emulsion of 0.2 cc. of medulla of rabbit dead of inoculation with the fixed virus of rabies mixed with 0.5 cc. of plain broth.	7	Short period of excitement followed by stupor; posterior incoördination; ascending paralysis; retraction of head; escape of much saliva from mouth; death in coma.	13	Meninges and trachea slightly congested. Bladder distended. Serum and broth cultures from meninges and heart's blood sterile after 48 hours incubation.
P. 86	1520	Same as No. P. 85 except 0.5 cc. of P., D. & Co's diphtheria antitoxine (500 units) used instead of broth. Emulsion inoculated with 1 loopful of a 40-hr. broth culture of B. diphtheriæ ½ hour before inoculation into rabbit.	7	Same as No. P. 85.	13	Same as No. P. 85.
P. 87 (control)	3050	Same as No. P. 85 except medulla of another rabbit (dead of fixed virus of rabies) used.	7	Same as No. P. 85.	12	Same as No. P. 85.
P. 88	2380	Same as No. P. 87 except 0.5 cc (500 units) P. D. & Co's diphtheria antitoxine used instead of plain broth. Emulsion inoculated 1 hr. before use with 1 loopful of a serum culture of a virulent B. diphtheriæ.	7	Same as No. P. 85.	11	Same as No. P. 85.
268 (control)	1300	In left subdural space with 0.2 cc. of emulsion of 0.2 cc. of medulla of rabbit No. 266 (original from street rabies, Case 33) made up with 0.5 cc. of plain broth.	13	Excitement followed by stupor; posterior ascending paralysis; retraction of head; much saliva escaping from mouth; death in coma.	15	Slight meningitis; trachea congested; bladder distended. Cultures from seat of inoculation and heart's blood sterile after 48 hrs. in incubator.

TABLE IV—*continued.*

No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days to onset of symptoms.	Symptons.	No. of days from inoculation till death.	Autopsy.
269	1420	In same manner, dose and medulla as with No. 268, but emulsion made by using 0.2 cc. of medulla to 0.5 cc. of P. D. & Co's diphtheria antitoxine (500 units). The emulsion was inoculated ½ hr. before use with 1 loopful of 40-hour broth culture of virulent B. diphtheriæ.	14	Slight excitement of animal noticed on day following inoculation which subsided within 18 hrs. No further symptoms till the 14th day, when began the usual course as observed in rabbits suffering from rabies.	16	Same as in No. 268, and in addition liver slightly congested.
270 (control)	2050	Same in every respect as No. 268, except that the medulla of rabbit No. 267 (mate to No. 266, original from street rabies, Case 33) was used.	19	Same as No. 268.	21	Same as No. 268.
271	2000	Same as No. 269 except same medulla as used with No. 270 and emulsion inoculated with 1 loopful of 24-hr. serum culture of virulent B. diphtheriæ ¼ hr. before use.	20	Three days after inoculation rabbit excited, showed some posterior incoördination; 24 hours later less excited, incoördination very slight; ate food freely. 72 hours later animal apparently perfectly well. No further symptoms until the 20th day after inoculation when began the usual course as in No. 268.	24	Same as No. 268, except small hæmorrhagic area—subdural—at site of inoculation.

virus had been added diphtheria antitoxine and virulent diphtheria bacilli, symptoms of slight cortical irritation were present on the first and third days respectively after trephining. These symptoms rapidly disappeared and the animals remained well until the expiration of the ordinary incubation period of street rabies when they exhibited the same train of symptoms as their controls, and after death gave the same autopsy findings.

The foregoing experiments demonstrate that the medulla of Rabbit No. 2 (dead 22 days after inoculation with an emulsion of the medulla of Mrs. R.) could not have contained the rabic virus, for if this virus had been present it would have manifested itself in Rabbits Nos. 6 and 7 which were inoculated with the medulla of Rabbit No. 2 combined with diphtheria antitoxine.

The question may, however, still be raised whether a combined infection with rabies and diphtheria may not have existed in the patient and that only the virus of diphtheria was transmitted to the rabbits inoculated with her medulla. As the ultimate findings could not be anticipated, the opportunity of determining the protective influence of diphtheria antitoxine upon the animals inoculated directly with the medulla of the patient was unfortunately lost.

Inasmuch as Roux * found that the medulla and cord of an animal inoculated 4 days before with fixed virus—that is, 3 days before the appearance of symptoms—were capable of producing rabies when inoculated into other animals, it seems most improbable that the virus of rabies should not, if it were originally present in the medulla of the patient, have been demonstrable in the medullæ of the rabbits inoculated with this medulla and dying 22 days after the inoculation.

In order to shed further light upon the question of the possibility of a mixed infection in the case of Mrs. R., the experiments recorded in Table V were undertaken to determine:

(*a*) How soon after subdural inoculation with the virus of rabies the medulla of a rabbit may contain sufficient virus to produce symptoms of rabies in other animals inoculated therefrom.

(*b*) The effect, if any, on the incubation period, course of symptoms, etc., in rabbits of inoculation with the medullæ of rabbits killed, during the incubation period of rabies, by subdural inoculation of B. diphtheriæ.

It will be noted from Table V that the medullæ of rabbits Nos. P103

* *Annales de l'Institut Pasteur*, 1889, iii, 77 and 1888, ii, 24.

SERIAL INOCULATIONS TO DETERMINE THE PRESENCE OF THE RABIC VIRUS IN RABBIT

First Set of Inoculations.							Sec		
No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days from original inoc. to onset of symptoms.	Symptoms.	No. of days from original inoculation till death.	Autopsy.	No. of rabbit.	Wt. in grammes.	Inocula
P102 (control)*	2020	In left subdural space with 0.2 cc. of emulsion of 0.4 cc. of medulla of rabbit No. P. 101 (dead from inoculation of fixed virus of rabies) rubbed up with 1 cc. of plain broth.	7	Short period of excitement followed by stupor; posterior incoördination; ascending paralysis; retraction of head; escape of much saliva from mouth; death in coma.	12	Slight congestion of meninges and trachea. Bladder distended. Serum and broth cultures from meninges and heart's blood remained sterile after 48 hours in incubator.			
P103	1900	Same as No P. 102, except that in addition 4 days later animal was given through original opening in skull 0.2 cc. of emulsion of 48-hour broth with a 48-h. serum culture of B. diphtheriæ.	5	Much excitement; posterior incoördination; ascending paralysis. (Excitement kept up longer than in rabies.) Death in coma.	7	Much congestion of meninges; no pus. Trachea congested. Serum and broth cultures from meninges showed B. diphtheriæ pure. Similar cultures from heart's blood remained sterile after 48 hours in incubator.	P116 P117	990 960	Same as No. P. ... emulsion made ... of rabbit ... up with pla... b... Same as No. ... emulsion made ... 0.2 cc. of ... No. P. 103 ... cc.) of P. D. ... antitoxine.
P104	1960	Same as No. P. 103, except second inoculation given 3 days after first.	4	Same as No. P. 103.	5	Same as No. P. 103.	P112 P113	930 880	Same as No. ... medulla of ... used for ... Same as No. ... 50 units P. ... ria antitox... making em...
P105	2470	Same as No. P. 103. except second inoculation given 2 days after first.	3	Same as No. P. 103.	4	Same as No. P. 103.	P110 P111	2200 1420	Same as No. ... emulsion made ... of No. P. ... Same as No. P. ... P. D. & Co.'s dip... ine used in mak...
P106	2190	Same as No. P. 103, except second inoculation given 1 day after first.	2	Same as No. P. 103.	2	Same as No. P. 103.	P108 P109	2200 1980	Same as No. ... sion made fro... No. 106. Same as No. ... P. D. & Co.'s dip... ine used in mak...
P107	2200	Same as No. P. 103, except 20-day broth culture of B. diphtheriæ used instead of plain broth in making emulsion.	1	Same as No. P. 103.	5	Same as No. P. 103.	P114 P115	940 900	Same as No. ... sion made ... No. P. 107 Same as No. ... P. D. & Co.'s dip... ine used in mak...

* This animal forms one generation in the series used in this laboratory for the perpetuation of the fixed vir... of r...

...ATED WITH THIS VIRUS AND SUBSEQUENTLY KILLED BY THE DIPHTHERIA BACILLUS.

...ET OF INOCULATIONS.					THIRD SET OF INOCULATIONS.						
...n	No. of days to onset of symptoms.	Symptoms.	No. of days from inoc. till death.	Autopsy.	No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days to onset of symptoms.	Symptoms.	No. of days from inoc. till death.	Autopsy.
...2 out with ...o medulla ...1 rubbed ...h	7	Same as No. P. 102, except shorter period from onset of symptoms till death. (This is almost always true with small rabbits.)	9	Same as No. P.102.							
...e ept with ...bbing up la f rabbit 50 nits (0.5 's phtheria	7	Same as No. P. 116.	9	Same as No. P.103.							
e ept with). P. 104 io	7	Same as No. P. 116.	11	Same as No. P.103.							
e ept that o. diphthe- re used in	7	Same as No. P. 116.	10	Same as No. P.103.	P120	1220	Same as No. P. 116, except medulla of No. P. 113 used in making emulsion.	7	Same as No. P.116.	10	Same as No. P.102.
					P121	1420	Same as No. P. 120, except 50 uts. P. D. & Co.'s diphtheria antitoxine used in making emulsion.	7	Same as No. P.116.	10	Same as No. P.102.
e ept with o medulla	7	Same as No. P. 102.	14	Same as No. P.102.							
ce 50 units e antitox- e ulsion.	7	Same as No. P. 116.	10	Same as No. P.102.							
xpt emul- n lulla of	7	Same as No. P. 102.	9	Same as No. P.102.							
ce 50 units er antitox- e ulsion.	7	Same as No. P. 102.	15	Same as No. P.103.							
ex pt emul- d a of No.	7	Same as No. P. 116.	9	Same as No. P.102.							
ce 50 units er antitox- ulsion.	7	Same as No. P. 116.	10	Same as No. P.102.							

...ad is noted here incidentally as a control in the inoculation of Nos. 103-107.

to P107, inclusive, dying from diphtheria infection 7, 5, 4, 2, and 5 days respectively after subdural inoculation with the fixed virus of rabies were found to contain the virus of rabies apparently unchanged.

Rabbits Nos. 108 to 117, inclusive, comprising the second series of inoculations from the above showed the same incubation period, train of symptoms and post-mortem lesions as control rabbits inoculated in the ordinary manner with the fixed virus of rabies. Though from each of the emulsions used in inoculating these animals (Nos. P108-P117) B. diphtheriæ was grown in pure culture, yet the organism was recovered from the brains of but half of them after death. Its presence or absence seemed not to be determined by the use or non-use of diphtheria antitoxine in making up the emulsions, nor by the length of time elapsing from inoculation till death.

Nos. P120 and P121, the only animals inoculated in the third set, gave the incubation period, train of symptoms, and autopsy findings of controls inoculated in the ordinary manner. Diphtheria bacilli were not recovered from their brains after death though the organisms were present in small numbers in the emulsion from which they were inoculated.

In the light of the experiments recorded in Table V it does not seem possible to doubt that if the virus of rabies had really been present in the medulla of the patient, it would have been transmitted in demonstrable form to the medullæ of the rabbits inoculated therewith, but, as has already been shown, such transmission did not occur, and the inference is, therefore, warrantable that the rabic virus was absent from the patient's medulla.

Subdural inoculations of B. diphtheriae.—Some experiments have been made in this laboratory and others are now in progress relating to the production of acute and delayed meningitis in rabbits by the subdural inoculation of pure cultures of B. diphtheriæ. The results of these, with report of tissue changes, are reserved until more extended observations have been made. One series, however, may be given here. B. diphtheriæ—nine generations removed from the original source—was grown 23 days in sugar-free bouillon and the following inoculations made:

Rabbit	8,	weight	2170 g.,	received	0.05 cc.	in	left subdural	space.
"	9	"	2080 g	"	0.1 cc.	"	"	"
"	10	"	2000 g.	"	0.2 cc.	"	"	"
"	11	"	1680 g.	"	0.3 cc.	"	"	"
"	12	"	1800 g.	"	0.4 cc.	subcutaneously	in	right

groin.

Nos. 9, 10 and 11 died within 24 hours. At the autopsies was found acute meningitis, and B. diphtheriæ was recovered from the point of inoculation. No. 12 died on the 4th day.

No. 8 remained somewhat stupid for two days and then became apparently normal until the 8th day, when it showed great excitement if irritated, whirling about rapidly and rushing around the room in a frenzied manner, this attack lasting about one minute. These phenomena could, with gradually decreasing readiness, be produced by stimulation during a period of 3 days, after which they subsided altogether. The rabbit then remained in apparent good health until the 23d day after inoculation, when its posterior extremities were noticed to be weak and slightly incoördinated. On the following day they were completely paralyzed. The paralysis rapidly ascended. 48 hours after the beginning of incoördination the rabbit was lying on its side with limbs extended, head retracted, and breathing spasmodically. At no time during its final sickness was there any excitement observed nor any marked escape of saliva from the mouth. It may be mentioned, however, that the symptoms were difficult to distinguish from those exhibited by two rabbits dying from inoculation with the fixed virus of rabies and one dying from "street rabies"—second generation from a rabid wolf—which were under observation at the time in the same room with Rabbit No. 8. The animal died on the 27th day after inoculation.

Autopsy. General condition of rabbit good. Meninges not congested. Portion of left cerebrum about 1 cm. in superficial diameter and extending, at seat of inoculation, down to ventricle, was markedly softened. Membranes of cord slightly congested. Liver deeply congested. Urinary bladder much distended.

Cultures. From the softened area of the cerebrum no growth was obtained; from the medulla a few colonies on serum of B. diphtheriæ; from the heart's blood a few colonies of staphylococci.

SUMMARY OF PATHOLOGICAL REPORT.

1. The bacillus isolated from the central nervous system of Mrs. R. was Bacillus diphtheriæ, and doubtless such was the identity of the bacillus discovered in the nerve cells of the pons and medulla.

2. The histological lesions, as far as they were observed in the central nervous system and kidney, were the same as those observed after death from ordinary clinical and experimental diphtheria.

3. The subdural inoculations in rabbits of portions of the medulla

of Mrs. R. produced symptoms simulating rabies in their time of onset and general character. The gross post-mortem findings in the animals, resembled in their negative character those of rabies, but B. diphtheriæ was isolated culturally from the central nervous system. Synchronous inoculation of diphtheria antitoxine protected rabbits against otherwise fatal doses of the emulsions of the medullæ of the first series of rabbits.

4. The mixture of diphtheria antitoxine, or of diphtheria antitoxine and diphtheria bacilli, with the virus of rabies is without influence upon the manifestations of rabies.

5. The medullæ of rabbits inoculated with the fixed virus of rabies and killed during the period of incubation, 2 to 7 days after the original inoculation, by subdural injection of cultures of the diphtheria bacillus, contain the virus of rabies in a form capable of transmitting rabies to rabbits inoculated in series with such medullæ.

6. The subdural inoculation of a small dose of pure culture of B. diphtheriæ, isolated from another source, produced symptoms which, in their time of onset and subsequent history, resembled in some degree those of rabies in rabbits.

I desire to express sincere thanks to Professor F. F. Wesbrook, under whose direction the above study has been conducted, to Dr. O. McDaniel and to Dr. E. Bates Block for their suggestions and criticism.

Review and Conclusions.

The history and the clinical symptoms in the case of Mrs. R. pointed toward the diagnosis of rabies. The well-authenticated history of a bite on the cheek by an unknown animal, the two months' incubation period, the onset with extreme pain and numbness in the region of the scar, the development of the characteristic laryngeal and respiratory spasms on attempting to take liquids, the spasms at first being slight, later most pronounced, and toward the close feeble or absent, the insomnia, the absence of fever in the beginning, which later in the disease became pronounced, the rapid pulse at all stages, the attacks of violent delirium interspersed with periods of calm and complete rationality, the absence of all symptoms pointing toward any other simu-

lating disease, and the fatal termination, all serve to make an almost complete picture of rabies.

One feature of the case not in harmony with the diagnosis of rabies is the long period over which the disease continued prior to the fatal termination. As is seen from the history, the course of the illness, dating from the onset with pain, sleeplessness and mental depression, lasted 14 days. If time is estimated from the onset of the first laryngeal spasm, ten days elapsed before the fatal outcome. On the other hand, most authorities place the death limit in rabies at five to eight days.

A second clinical feature manifested in this case which does not appear to correspond to the usual symptom-complex in rabies is the presence of a large amount of albumin and of casts in the urine. Most of the clinical writers make no mention of such a complication in rabies. Roger,* however, speaks of the occurrence of dysuria, albuminuria and glycosuria in rabies, and he cites Samson and Chippindale as having noted hæmoglobinuria and the presence of casts.

The gross pathological findings likewise seemed to confirm the clinical diagnosis, since the post-mortem examination revealed no lesions aside from mild cerebral congestion. But the value of such confirmation is slight, in view of the fact that no characteristic gross lesions of rabies have hitherto been determined by any observer, and a confirmation based on negative pathological conditions is of doubtful value. The microscopical changes found in the nervous centres, although definite, are not of decisive diagnostic significance.

An unexpected and important discovery was the finding of the Klebs-Löffler bacillus in the ventricular fluids and in the tissues of the medulla oblongata of the patient. The presence of the bacillus in microscopical sections confirmed the cultural results, and especially to be emphasized is the demonstration of characteristic diphtheria bacilli within nerve cells. The determination of the identity of the bacillus with the genuine Bacillus diphtheriæ was so complete that there can be no room for doubt upon this point. The bacillus was of

* Roger, Article "Rage" in Charcot, Bouchard and Brissaud's Traité de Médecine, T. i, p. 608. Paris, 1891.

ordinary virulence and produced toxine and led to the generation of antitoxine identical with diphtheria toxine and antitoxine.

Not less suggestive of rabies than the clinical history of the patient were the results of subdural inoculations of rabbits with emulsions prepared from the medulla of the patient. There occurred the long period of incubation (20 and 21 days) followed by phenomena similar to those in experimental rabies of rabbits, and the rabbits inoculated subdurally with the medullæ of the first rabbits behaved in a similar manner.

But here again the Bacillus diphtheriæ, which was present in the medulla of the patient, was demonstrated with similar distribution in the rabbits' medullæ.

Of decisive significance in the interpretation of the case is the demonstration that the mixture of the emulsion of the medulla of the first series of rabbits with diphtheria antitoxine, as well as the injection of antitoxine followed by inoculation of the medulla, prevented the appearance of any abnormal symptoms after subdural inoculation, whereas in control experiments diphtheria antitoxine manifested no appreciably protective influence as regards either the fixed virus of rabies or the virus of street rabies. It is, therefore, justifiable to infer that the virus of rabies was not present in the medullæ of the rabbits inoculated directly with the medulla of the patient, for otherwise it should have been capable of demonstration after the neutralization of the diphtheria toxine by antidiphtheritic serum. Although the opportunity to mix diphtheria antitoxine with the medulla of the patient was lost, the experiments recorded in Table V justify the conclusion that if the virus of rabies had been present in combination with the diphtheria bacillus in this medulla it would have been transmitted in demonstrable form to the medullæ of the rabbits inoculated therewith and dying 22 days afterward. As such transmission is excluded by the results of the inoculation of Rabbits Nos. 6 and 7, the absence of the virus of rabies from the patient's medulla must be regarded as established.

We are forced, therefore, to the conclusion that the case reported in this paper was one of infection of the central nervous system with

the diphtheria bacillus and not one of rabies, and this conclusion is confirmed by the experimental reproduction of a similar localization with a prolonged period of incubation by the subdural inoculation of a rabbit with 0.05 cc. of a 23 days' bouillon culture of the diphtheria bacillus (p. 472).

The portal of entry of the bacillus was not determined. There was no positive evidence of diphtheria in the throat, although the existence of the diphtheria bacillus in this situation was not excluded. The possibility that the patient was inoculated with the diphtheria bacillus through the wound and that it traveled along the nerves to the nervous centres, in the manner of the rabid virus, may be suggested, but of this there was no proof.

The close simulation of rabies both clinically and experimentally (a simulation all the more remarkable on account of the previous history of a bite on the cheek, followed by the usual period of incubation of rabies) by cerebral infection with the diphtheria bacillus is certainly most curious and interesting. We have abundant evidence that the cerebral localization of various infectious agents, such as those of tuberculosis, of tetanus, etc., may be attended by morbid phenomena very unlike those of the ordinary localizations of the same agents elsewhere in the body, and our case demonstrates that the same may be true for the diphtheria bacillus.

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AN EXPERIMENTAL INVESTIGATION OF THE TREATMENT OF WOUNDS OF THE HEART BY MEANS OF SUTURE OF THE HEART MUSCLE.

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PART I.

INTRODUCTORY.

In attempting to trace the history of the growth of knowledge regarding wounds of the heart, the investigator is soon impressed by the infrequency with which this subject is mentioned by older writers. In the works of Hippocrates and Celsus we find occasional mention of wounds of the heart, always, however, accompanied by the statement that such wounds are followed by immediate death. Paul of Ægina, Roland, Lanfranc give short descriptions of the symptomatology of cardiac wounds, and all agree that death results at once. Toward the close of the fifteenth century this opinion was still prevalent. The beginning of the sixteenth century, however, saw the dawn of a new era. Foreign bodies, most often bullets and arrow-points, had been found healed in the hearts of living animals. Reports were published which claimed that deer, wild boar, and other animals of the chase had, after receiving a wound of the heart, continued to run for minutes and even for hours. Soldiers who had been wounded in the heart were reported to have continued to fight in the excitement of battle for some minutes before death had overtaken them.

At about this period, Ambroise Paré published a report of the now

celebrated case of the Turin gentleman, who, although wounded in the heart during a duel, was able to pursue his antagonist 700 feet before he dropped to the ground and died. The learned societies of those days discussed this case with eagerness and commented on the rapidity of onset of the fatal symptoms after wounds of the heart. As evidence of this one finds occasional short papers on the subject in the medical literature of the seventeenth and eighteenth centuries. The writers of these papers advanced no new theories or new facts, and hence attracted little attention. They regarded these cases of delayed death after cardiac injury either as apocryphal or as mere curiosities of medical literature. It was only in the beginning of the nineteenth century that observers evinced a keener interest in the subject, as is shown by the more comprehensive investigations of Jamain, Sannetti, and others, published at this period, and it remained for G. Fischer to lay the foundation for all future work on this subject by the publication of his exhaustive and critical study of 452 cases of wounds of the heart in man, collected from medical literature.

The main results arrived at by Fischer * may be summarized in brief in the following: Seven to ten per cent of all wounds of the heart heal and the patients recover. In the cases with a fatal outcome, death sometimes occurs simultaneously with the infliction of the injury, more frequently, however, it is delayed a few moments, or less often for hours or days. Wounds of the ventricles occur with much greater frequency than do those of the auricles. Wounds of the right ventricle are more frequent than those of the left (in the ratio of 27 to 22), because in man the right ventricle forms the greater part of the anterior surface of the heart, while the left ventricle is brought forward only during each systole. On the other hand, the death rate after wounds of the auricles is higher than after those of the ventricles, and in this respect the right auricle predominates over the left.

Loison † has recently published a paper, based upon the reports of 277 cases of wounds of the heart in man, which he has collected from the medical literature of the last thirty years. Most of Loison's conclusions agree with those of Fischer. In 39.7 per cent of the patients the right, and in 33.3 per cent the left ventricle was injured. The mortality after wounds of the right was much greater than that after wounds of the left ventricle. In a large proportion of the cases, the fatal outcome was hastened by the over distension of the pericardial sac by the

* G. Fischer, Langenbeck's *Archiv*, 1867, ix, 571.

† Loison, *Revue de Chirurgie*, 1899, Nos. 1, 2, 3, et seq.

blood. Loison declares that the mortality rate varies considerably with the situation, the size and the character of the wound. In general, 85 per cent of all cardiac wounds prove fatal.

It may be said that the gravity of the case depends upon the size of the wound and its location. This does not mean, however, that wounds of small size, *i. e.* punctured wounds, bullet-wounds, are not attended with great danger. Indeed, in many cases these have been shown to cause death sooner than larger wounds. There are a number of factors to be considered which will be referred to later on, but the general statement—the larger the wound of the heart muscle, the more serious the prognosis—will be found to be applicable to most of the cases.

Does death ever occur at the same moment that the heart is wounded? Undoubted cases of this kind have been reported where the whole heart was torn off from the great vessels at its base. If the injury be not so severe as this, however, have we sufficient evidence to warrant the belief that the heart may stop beating at the same moment it is injured? In former times such cases were believed to be of very frequent occurrence. Even Fischer thought he had found reports of several undoubted cases. After having made a careful study of the cases collected by Fischer, I have become convinced that we must exclude most of them from this category, because in the main the reports contain few details and hence are untrustworthy. In only one case, that of the famous soldier L'Atour D'Auvergne,* is the report of the injury and death so clearly told that there can be no doubt of the simultaneous occurrence of the cardiac wound and the soldier's death.

The older writers, as was stated above, believed that death followed quickly after wounds of the heart. They explained this on the assumption of an influence of some kind, reflex or otherwise, upon the nerves or nervous centres of the heart. At the same time, however, they disagreed about the source and character of this innervation, and their successors of to-day have a similar contention. At one time the belief was prevalent that the stimuli for the contraction of the heart passed to the cardiac muscle through the sympathetic ganglia. These ganglia are very numerous in the septum between the two auricles. The experiments on rabbits and dogs by Kronecker and Schmey † showed that there was a spot in the septum between the ventricles of these animals where puncture with a fine needle was followed by immediate stoppage of the heart. They inferred that this was due to some injury to the large nerve-ganglia

* See Fischer, loc. cit. Case 72.

† Kronecker and Schmey, *Sitzungsberichte d. Berliner Akad.*, 1884, p. 87.

in this region and named this spot the "coördination centre." Kronecker and Schmey declared that in all probability a similar spot exists in the human heart. The investigations of Krehl,* Romberg,† W. His,‡ and Schaefer § gave conclusive proof that these nervous ganglia were derived from the sympathetic and that they could, therefore, carry only centripetal or sensory and no motor stimuli. His believes that the heart contains only sensory nerves, while Romberg calls attention to the fact that the fœtal heart contracts rhythmically long before either nerves or nervous ganglia can be found in the cardiac muscle. It is incorrect, therefore, to attempt to explain the cardiac stoppage in the experiments of Kronecker and Schmey, and likewise the possible sudden arrest of the heart after wounds of that organ, as a result of injury to the nerve ganglia in the heart. Engelmann ‖ has shown that it is possible to incise the heart-muscle in such a manner that, while the continuity of the muscle is not totally disturbed, all the nerves are doubtlessly cut across. Nevertheless, muscular contractions continue. Porter¶ concludes that nerve cells, if present, are not essential to long-continued, rhythmical, co-ordinated contractions of the ventricle, and that the causes of rhythmic contractions of the ventricle lie in the ventricle itself.

If there are, therefore, no motor nerves in the heart, and if this organ can contract after all the sensory nerves have been divided, there must be a true automatism of the heart-muscle. This is the prevailing opinion among writers of the present day. Investigators are undecided as to the explanation of the so-called co-ordination centre of Kronecker and Schmey. In man, wounds of the septum ventriculorum, in the region that would correspond to the co-ordination centre in animals, are very rare indeed. It is only on this assumption, however, that we can explain the immediate cardiac arrest and death in the case of the Chevalier L'Atour D'Auvergne. As no other undoubted case of this kind has been reported we must conclude that immediate cardiac asystole and death after wounds of the heart in man, unless indeed the whole organ be torn from its attachment, is excessively rare.

When death is delayed after an injury of the heart, what is the cause of the stoppage of this organ?

It must, of course, be understood that in the following considerations

* Krehl, *Deutsche med. Wochenschr.*, 1889, p. 549.

† Romberg, ibid., 1890, p. 440.

‡ W. His and Romberg, *Archiv f. exp. Path. u. Pharm.*, 1892, xxx, 51.

§ Schaefer, *Verhandl. d. IX Congress. f. innere Medecin*, 1890.

‖ Engelmann, *Archiv f. d. ges. Physiologie*, 1896, lxv, 119; 535.

¶ Porter, *Journal of Experimental Medicine*, 1895, i, 319.

no reference will be made to the secondary or complicating causes of death such as sepsis, shock from the severity of the injury and the like, for they have no direct bearing on the subject in hand. There remain for consideration: 1. The effect on the heart of the loss of a large amount of blood; 2, the change in the character of the heart's action as the escaping blood fills and distends the pericardial sac.

That the hæmorrhage from a wound of the heart is usually very great and often sufficient to cause death in a few seconds needs no explanation. Clinical experience has long ago shown that, after the loss of a large quantity of blood, the heart suffers mainly because it has no longer sufficient fluid to "work on." For the blood, circulating through the heart chambers, stimulates the heart-muscle to contract, and when there is little or no fluid in these cavities, the rhythmic contractions and relaxations of the heart-muscle soon cease. The larger the wound and the freer its communication with the surface of the body, the more rapidly will cardiac asystole and death supervene. The same is also true where the heart wound communicates with one or the other pleural cavity into which the heart can pour the blood from the entire body.

If the wound in the heart-muscle, however, permits of the free escape of blood, while the wound of the parietal pericardium is either very small as compared with that in the heart wall, or the pericardial rent becomes closed or partially closed by clots or by the pressure of the soft parts around it, the blood will be unable to escape from the pericardial sac and the latter will thus become more and more distended. The intrapericardial pressure rapidly rises and the pressure exerted upon the heart by the blood in the pericardium becomes very much increased. To this condition Rose,* although he was not the first to call attention to its importance, gave the name of "heart tamponade." Morgagni † had already recognized its danger. Cohnheim ‡ investigated the subject in numerous experiments on dogs. He injected various quantities of fluids into the pericardial sac of the animals and showed that, with the rise of the intrapericardial pressure, the heart's action steadily grows weaker. A point is soon reached where the heart stops beating altogether. Cohnheim demonstrated that the pressure of the fluid in the pericardial sac is mainly expended upon the auricles and the great vessels at the base of the heart. While the ventricles still contract, the auricles and the great vessels are compressed, the entry of blood into

* Rose, *Deutsche Zeitschr. f. Chirurgie*, xx, 329.

† Morgagni, De Sed. et Caus. Morborum, Epist. 69, Sect. 5.

‡ Cohnheim, *Allgem. Pathologie*, i.

the heart grows less and less, the heart pumps itself dry and finally the contractions of the ventricles also cease. In other words, while the causes are different, the results are the same as in death from acute hæmorrhage, *i. e.* the heart ceases to beat because there is no more fluid upon which it can contract. Rose and many writers after him believe that this heart tamponade is in many cases the chief cause of death.

Dyspnœa, cyanosis, small thread-like pulse, the physical signs of distension of the pericardial sac with fluid, together with the characteristic situation of the external wound, the hæmorrhage, and the symptoms of collapse, are the symptoms that are generally described. Some writers have attributed considerable value as a diagnostic sign to a peculiar whirring noise to be heard over the lower part of the heart; but Riedinger,* Rose,† Delorme,‡ Loison,§ consider it as neither constant nor characteristic.

While injuries of the heart are most often followed by death before medical aid can arrive, there are some cases in which the patient survives for a number of hours or days. The treatment of these patients has consisted in general and local measures such as cleansing and packing the external wound, absolute rest, the application of cold to the præcordial region, the administration of morphine and other narcotics, sometimes venesection (Dupuytren, Stromeyer, Rose). If the hæmorrhage continues and the patient grows worse, the case has been considered a hopeless one.

The first step toward a more direct and local treatment was taken by Rose when he recommended that the pericardial sac should be punctured through the fourth left intercostal space, in order to relieve the dangerous symptoms of heart tamponade. If this did not suffice and the dangerous symptoms continued, the pericardial sac should be opened and all clots and blood removed from it. While in some cases the good results of this operation cannot be denied, it seems to me that Rose goes too far when he declares that "the opening of the pericardium and the immediate removal of the heart tamponade deserves to have as important a place in surgical procedure as tracheotomy."

In man, puncture of the pericardial sac and pericardotomy is already a recognized procedure in surgical practice. In the large majority of cases, the pericardial sac can be exposed and opened without injury to the

* Riedinger, Krankheiten des Thorax, *Deutsche Chirurgie*, xlii, p. 180.

† Rose, loc. cit.

‡ Delorme, *Chirurgie de guerre*, 1893.

§ Loison, *Revue de Chirurgie*, 1899, Nos. 1 and 2.

left pleura. But the technique of the operation needs further development, and the anatomical landmarks in normal and abnormal conditions must be more clearly defined. The recent investigations of Voinitch-Sianojensky* and of Delorme and Mignon† constitute a distinct advance in this direction.

It would be manifestly beyond the scope of this paper to describe the various methods proposed for the operations of paracentesis pericardii and of pericardotomy. For these, the reader is referred to the short but comprehensive work of Terrier and Reymond, *Chirurgie du Coeur et du Péricarde.* There are several points, however, to which we would draw attention. Up to within the last few years the belief was widespread that large collections of fluid in the pericardium push the heart backwards against the vertebral column. If this were always true, there would be little danger of wounding the heart during an aspiration unless the aspirating-needle were made to penetrate too deeply. Recent investigations have shown that this belief is not always true. Large effusions into the pericardial sac may and often do push the heart forward against the anterior wall of the chest, in which situation it might easily be injured. The larger the quantity of fluid in the pericardial sac, the more is the heart pushed forward. This has been seen in the human subject by Rehn;‡ experimentally, in the cadaver, by Delorme and Mignon§ and Voinitch-Sianojensky;|| and in the living animal body by myself.¶ If there are adhesions between the heart and the anterior wall of the pericardial sac, the injury of the heart during paracentesis of the sac may also take place. Kümmel** found adhesions between the heart and the parietal pericardium 2 centimeters above the point where he had aspirated the pericardial sac. Numerous cases have been reported where the heart was actually injured during this procedure. Hence Eiselsberg†† declares, as a result of his experience, that the danger of paracentesis is often greater than that of incision of the pericardial sac. He recommends that in pericardotomy the pericardial sac, when exposed, should be sewn to the edges of the wound in the chest wall,

* Voinitch-Sianojensky, *Archiv. Russ. de Chirurgie*, St. Pétersbourg, 1897, ii; also *Revue de Chirurgie*, 1898.

† Delorme et Mignon, *Revue de Chirurgie*, 1895, pp. 797; 987, and 1896, p. 56.

‡ Rehn, Langenbeck's *Archiv*, 1897, lv, 315.

§ Delorme and Mignon, loc. cit.

|| Voinitch-Sianojensky, loc. cit.

¶ See Exps., vi-vii-viii, p. 513.

** Kümmel, see Eiselsberg.

†† Eiselsberg, *Wiener klin. Wochenschr.*, 1895, No. 2.

thus protecting the incision in the soft parts, and the pleural cavities against injury and infection.

While paracentesis or pericardotomy in the treatment of heart tamponade is based on rational grounds, there is at the same time a noteworthy risk connected with its performance. When the pressure on the heart has been removed, the hæmorrhage from the wound in the heart-muscle is apt to begin anew, or, if it had not ceased, to become very much larger. For these reasons, surgeons have often hesitated about following Rose's recommendations, and have sought for other and more efficient methods of stopping the hæmorrhage from a wound of the heart.

Many years ago Billroth declared that no surgeon, who wished to preserve the respect of his colleagues, would ever attempt the suture of a wound of the heart. In 1884 Riedinger* made the oft-quoted statement that "the proposition to suture a wound of the heart, although made in all seriousness, needs hardly a mention." Tillmanns declared in one of the recent editions of his book that surgery is powerless when confronted with a hæmorrhage from the heart-muscle. There were others (König,† Delorme,‡ etc.), however, who dared to give voice to their opinion that suture of a wound of the heart, though difficult and connected with great risks, was theoretically and probably technically possible. On logical grounds one might ask why direct surgical treatment of the wound, according to the general principles of wound treatment, was never proposed and attempted. For had it not been proven centuries ago that cardiac wounds could heal and that foreign substances could heal in the heart-muscle without interfering with its function? Had not the heart been exposed in the operation of pericardotomy and were writers not agreed that this could be done with comparative safety by the use of modern aseptic technique? In answering this question, we must remember that we have to deal with an organ of first importance which is in constant motion, and which, moreover, was believed to be very sensitive to the smallest mechanical insult or injury. It was feared that during the slightest manipulation the heart might suddenly stop, that the mere passage of a needle might be followed by the direst results. Hence cardiac suture was until very recently considered an unwarrantable surgical procedure.

With the growth of such ideas as those of König and of Delorme, investigators turned to the animal heart to gain knowledge and experience on this subject.

* Riedinger, loc. cit.

† König, *Lehrbuch d. Chirurgie*, ii.

‡ Delorme, *Chirurgie de Guerre*, 1893.

Rosenthal,* I believe, was the first to attempt to treat a wound of the heart in an animal by direct means. Early in 1895 he showed to the Medical Society of Berlin a dog whose heart, after resection of the sternum, he had wounded and tamponed with iodoform gauze. The wound healed and the animal survived. Del Vecchio† reported that he was successful in keeping alive a dog that had suffered two perforating wounds of the left ventricle and the subsequent operation of suturing. Salomoni‡ twice wounded and successfully sutured the canine heart. In his cases, after 15 to 20 days, the dogs were killed and the wounds were found healed. About one year later F. Bode§ made a series of experiments on rabbits and published a careful and detailed account of his observations. He made small wounds in the ventricles of the rabbit's heart and then closed these with fine silk sutures. The conclusions he arrived at agree almost entirely with some of Fischer's statements concerning the human heart. In none of Bode's experiments did the heart stop suddenly after the wound had been made nor did the passage of the needle and suture and the tying of the latter ever cause more than a temporary irregularity of the heart's action. He found that wounds of the right ventricle bled more freely and were more difficult to manage than those of the left. Wounds of the auricles were very dangerous and almost always resulted in the death of the animal from hæmorrhage before the suture could be applied. Every time Bode passed a needle and suture through the heart-muscle he observed that the next systolic contraction was somewhat delayed, and that there followed a short period of arhythmical heart's action; nevertheless the heart soon recovered its regular movements. The wounds which he made were very small, however, and the sutures he applied few in number. He states that he is convinced that many of his results can properly be applied to the human heart.

When we turn to the human heart, we find that suture of the cardiac would has thus far been done in nine cases. Some of the reports are very short, and contain few details. Cappelen‖ sutured the heart-muscle of a patient with a non-penetrating wound. The patient died 2½ days later from secondary hæmorrhage of the coronary artery. Farina¶ of

* Rosenthal, *Deutsche med. Wochenschr.*, 1895, No. 2.

† Del Vecchio, *Riforma med.*, 1895, ii, No. 79; also *Centralbl. f. Chirurgie*, 1895, p. 574.

‡ Salomoni, *Centralbl. f. Chirurgie*, 1896, No. 51.

§ F. Bode, *Beiträge z. klin. Chirurgie*, 1897, xix, 167.

‖ Cappelen, *Deutsche med. Wochenschr.*, 1896, p. 186.

¶ Farina, *Centralbl. f. Chirurgie*, 1896, p. 1224.

Rome closed a penetrating wound of the left ventricular wall by two silk sutures. The patient died of pneumonia one week after the operation and at the post-mortem examination the wound was found firmly healed. At the XXVI Congress of the German Surgical Society held in Berlin, April, 1897, Rehn* reported a successful case of suture of the heart. This occurred in a florist, 22 years of age, who, after being stabbed in the left side of the chest, fell unconscious. After three hours he revived, dragged himself along the ground for about 500 feet and again fainted. Shortly afterwards he was brought to the hospital in a condition of severe shock. In the fourth left intercostal space, three finger-breadths from the parasternal line, there was a stab-wound about one inch long. Cardiac dulness was increased towards the right; dyspnœa was extreme and cyanosis marked. The following morning his general condition was somewhat improved, although he was still suffering from shock. Towards noon of the same day he became very much worse, the dyspnœa and cyanosis had increased, the heart-dulness had extended still further to the right and to the left, and the patient appeared moribund. Rehn having decided to operate made an incision in the fourth left intercostal space and resected part of the fifth rib. The pericardial sac could now be seen. When the left pleura was opened, a large quantity of blood escaped from it. On opening the pericardial sac, a penetrating wound 1½ centimetres long, bleeding freely, was found in the wall of the right ventricle. The edges of the wound were approximated by three sutures. Although the case was complicated by an empyema of the left pleura, the patient recovered and remained well.

Parrozzani† published the report of a patient who received a wound so large that "the little finger could be passed into the ventricular cavity" in the left ventricular wall near the apex of the heart. The wound was closed by two deep and two superficial sutures, and the pericardial sac by six sutures. The wounds healed by primary union and the patient recovered.

In a second case operated on by the same surgeon, a woman received a stab-wound through the left ventricle, which was closed with two silk sutures. The patient died of exhaustion on the second day after operation, but at the necropsy the heart-wound was found firmly closed.

Parlavecchio‡ reports a remarkable case. The patient, a young man,

* Rehn, loc. cit.

† Parrozzani, *Bull. d. r. Acad. Med. di Roma*, 1897-8, xxiii, 243; also *Semaine med.*, 1897, No. 23.

‡ Parlavecchio, *Riforma med.*, 1898.

was able to walk to the hospital, a distance of one-quarter of a mile. He had a wound in the left intercostal space from which blood escaped during each systole of the heart. There was a left pneumo-hæmothorax and heart-dulness very much enlarged; the pulse was weak and irregular and the patient was pale and gasped for breath. Eight hours after admission the heart was exposed and a V-shaped penetrating wound 3.5 centimetres long was found in the wall of the left ventricle. The wound was closed with four interrupted silk sutures. The patient made an uneventful recovery and was discharged cured five weeks after operation.

Ninni* reports the case of a man, 33 years of age, who was stabbed in the chest with a short knife. After walking about 200 steps the patient fell to the ground and was brought to the hospital. He had a wound in the fifth left interspace in the mammary line. No radial pulse could be felt, although the heart was beating tumultuously. The patient remained unconscious and the heart's action became steadily weaker, although energetically stimulated. Without anæsthesia, an incision was made in the fifth interspace from the sternal margin to the mammary line, a second incision parallel to the first in the third interspace, and a third incision connecting these two along the sternal margin. The left pleura was incised and a large quantity of clotted blood allowed to escape from it. The pericardial wound, which was 3 cm. long, was enlarged. In the anterior wall of the left ventricle near the apex was found a wound 25 mm. in length, from which fresh blood was escaping. The wound was closed with two silk sutures and the pericardium by a continuous suture. While the left pleural cavity was being cleared of clots and before the cutaneous flap was adjusted, the patient died.

Pagenstecher† operated upon a young man, 17 years of age, who was stabbed just under the left nipple with a knife. After walking a few steps he fainted and was brought to the hospital, where he soon regained consciousness. Next morning he was operated upon, 6 cm. of the left fifth costal cartilage were resected, the wound in the pericardium was found and enlarged. Three cm. from the apex of the heart there was a wound in the wall of the left ventricle 3½ cm. long. A small stream of bright red blood issued continuously from the wound. After considerable difficulty sutures were introduced and the hæmorrhage was controlled. The patient made a complete recovery.

Suture of a wound of the auricle has been performed, and with relative

* Ninni, *Giornale internaz. d. Sc. med.*, 1899, No. 1.

† Pagenstecher, *Deutsche med. Wochenschr.*, 1899, No. 31.

success, in one case by Giordano.* The external wound was in the third left interspace in the anterior axillary line. After the third and fourth costal cartilages had been resected, a wound two centimetres long was found in the right auricular wall. This was closed by four superficially passed sutures. Nineteen days later, the patient died of empyema, having given no symptoms which pointed to lesions of the heart. At the autopsy the cicatrix of the wound in the auricle could be found only with difficulty.

Thus, of the foregoing nine cases of suture of the human heart, four patients recovered entirely; one case died of a complicating empyema nineteen days after operation, another died of pneumonia one week after operation, a third died from shock before the operation was completed. The other two patients lived for two days and died, the one from a secondary hæmorrhage from a coronary artery, the other from exhaustion.

PART II.

EXPERIMENTAL INVESTIGATIONS.

As a contribution to the development of this branch of surgical procedure, it seemed to me of value to attempt to determine how extensive an injury the mammalian heart could safely withstand, and how extensive a suture could be applied to the heart without interfering with its function. With this purpose in view, the following experiments were made on the heart of the rabbit, although I sometimes, for comparison, examined the heart of the dog. I chose the rabbit for most of the experiments, because in this animal one can expose the heart without opening the left pleural sac. The results obtained in the different animals agreed in all essential particulars.

Under ether anæsthesia, which was employed in all of the experiments, the 3rd, 4th and 5th ribs were exposed by a longitudinal incision through the skin and the costal cartilages cut close to the left border of the sternum. These cartilages could with care be dissected from the muscles around them and reflected outwards. The internal mammary artery was drawn outwards and the triangularis sterni muscle divided near its insertion. In this manner the whole of the anterior surface of the rabbit's pericardium could be exposed. The pericardial sac was then

* Giordano, *Riforma med.*, 1898, Sept. 9 and 10; *Semaine med.*, 1898, p. 407.

sewn to the thoracic muscles around the edges of the wound and then opened by a longitudinal incision. The heart was thus exposed and could be manipulated without danger to the left pleura. In making use of this procedure, I had followed the precepts laid down by Eiselsberg and Gussenbauer in performing pericardotomy in man. I found this procedure very valuable, for I was able thus to avoid almost uniformly the injuries to the pleura which occurred in so many of Bode's experiments, and to which the death of a large number of his animals was attributable.

When the experiments and observations on the heart of the animal had been made and the wound in the wall of the thorax was ready to be closed, the cartilages were returned into place, the cut end of the left pectoralis major muscle sewn to the insertion of that of the right side, the serous surfaces of the outer pericardial layer thus brought together, and by this means the pericardial sac again closed. The skin wound was then closed by interrupted silk sutures and an iodoform collodion dressing applied.

Most of the animals recovered rapidly from the operation. One to two hours after the experiment they were running about as if nothing had been done to them. Every one to two days exact notes were taken as to their condition, character of their hearts' action, etc.

The experiments and the results obtained I have arranged under three headings:

Series I. Wounds were made of various sizes and in various parts of the heart. No suture was applied to the cardiac muscle.

Series II. Suture of the normal intact wall of the heart.

Series III. Suture of the cardiac muscle after wounds had been made in it.

Series I.

WOUNDS OF THE HEART—THEIR SIZE AND SITUATION. THE HÆMORRHAGE. THE PHYSIOLOGICAL IRRITABILITY OF THE HEART-MUSCLE.

The heart was exposed in the manner above described and manipulated in various ways. The organ could be lifted well up into the thoracic wound without causing any appreciable change in the heart-beat. It could even be drawn forward so far that the greater part of the ventricles lay outside of the thorax, so that all sides of the ventricles could be thoroughly examined. When part of the heart-

muscle was grasped and gently compressed with a forceps, no appreciable difference in the character of the heart's action was observable. When, however, firmer pressure was made, a peculiar and characteristic irregularity was noticed. This irregularity was also to be seen when the heart-muscle was punctured with a coarse teasing needle. Every time the needle penetrated the heart-wall, there was a delay in the occurrence of the next systolic contraction, followed by a short period of very irregular action of the heart. This irregularity was usually noticeable during the time the needle was penetrating the epicardium and the moment that the endocardium was pierced. After a few moments the heart's action becomes regular again.

Several times, in order to determine just when the arhythmia

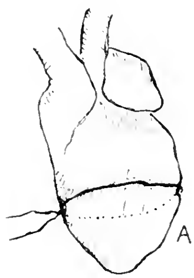

FIG. 1.
Transverse Ligation of Ventricles.

occurred, I proceeded in the following manner. I had noticed in previous experiments on the rabbit's heart that it was possible to tie a ligature transversely around a portion of the ventricles without more than temporarily disturbing the heart's action. (See Fig. 1.) Though the ligature was drawn very tightly, the part (A) below the ligature continued to beat, though somewhat more weakly than the rest of the ventricles.

When the ligature was tied, there was only a temporary irregularity of the heart lasting from two to five minutes. If anything less than one-half of the ventricles was thus tied off, the heart's action remained strong and regular. When the ligature was removed, the heart's action became very rapid, there being often 300 beats to the minute. In less than five minutes, however, the beats became slower

and soon returned to the normal. If more than one-half of the ventricles was tied off in this manner, the action of the heart at once became weak and very irregular and, unless the ligature was at once removed, stopped in less than one minute.

I tied off a part of the ventricles in this manner, and made a large incision penetrating into one ventricular cavity. On account of the ligature around the ventricles above the line of incision, there was no hæmorrhage. When the heart's action had returned to the normal, a needle was pushed through the heart-wall near the line of the incision. It was forced forward very slowly, so that the heart-muscle was penetrated layer by layer. The exact moment when this needle penetrated the epicardial layer and, likewise, when it passed through the endocardium, could be determined. While the needle passed through the epicardium the cardiac action was very irregular. Although at first I thought that the heart sometimes omitted a beat, I soon observed that the irregularity was due to a delay in each systolic contraction of the ventricles. With the needle in this position, and supposing that the irregularity would persist, I waited, but found that it disappeared in less than one minute. When I then pushed the needle slowly forward through the myocardium, the heart continued to beat regularly until the needle reached and passed through the endocardium. Then each systole again "lagged behind." Although the needle was left in place, passing through the entire heart-wall, the action of the heart became regular in less than one minute and remained so. I consider it noteworthy that the heart did not omit a single beat during the whole of this experiment.

This irregularity occurs also when the auricles are similarly punctured. As the walls of the auricles are very thin, their different layers cannot be injured separately, and, therefore, the exact moment when the arhythmia occurs cannot be determined. I was much surprised to find in two of my animals, however, that this irritability of the epicardium and endocardium was altogether wanting. Bode believed that such cardiac arhythmia occurred only during injury of the endocardium. My observations seem to show that both epicardium and endocardium react in this characteristic manner.

Heitler,* in his investigations on the electrical excitability of the heart-muscle, arrived at results which substantiate mine. He found that the passage of a weak galvanic current through the epicardial and the endocardial layers of the heart-wall caused an irregularity of the heart's action, while the myocardium did not react to the electric current.

The hæmorrhage that occurs from needle punctures of the heart in the animals experimented on is always small when the ventricles are injured, but more considerable when the needle is made to penetrate the auricular wall. In the latter case, however, as well as in punctured wounds of the ventricles the hæmorrhage soon ceases. Even when the needle is passed through the whole thickness of the heart, thus traversing the walls of both ventricles and the interventricular septum, the hæmorrhage was never so great as to endanger the animal's life. If the animal be killed 3 or 4 days after the experiment and the heart be then examined, the point of puncture can scarcely be seen as a slight depression on the surface of the heart; after one week it is impossible with the naked eye to find the point.

In one animal the heart was punctured in various directions a number of times. The hæmorrhage from the wounds was controlled by compression with gauze and the animal recovered.

The range of experimentation was enlarged by incisions in the cardiac muscle in various directions made by a scalpel with a blade one millimetre in breadth. It is a fact, so well known that it needs no comment, that the heart of the rabbit can be made to beat very slowly by allowing the animal to inhale a few drops of chloroform. By means of this expedient, it was possible for me to inflict the wounds with my scalpel during the systole or the diastole of the heart at will. Wounds that are made during the systole of the injured part bleed more than those made during the diastole. For a wound that is made during systole becomes larger with the diastolic relaxation of the heart-muscle, while, vice-versa, a wound that is made during the diastole becomes smaller with each systole. Thus a wound of a ventricle 2 mm. long, made in systole, would be almost 3 mm.

* Heitler, *Wiener klin. Wochenschr.*, 1898, Nos. 3 and 8.

in size during the diastole; and one of 2 mm. made in the ventricular wall during a diastolic relaxation, would become almost as small as one millimeter during the systolic contraction.

The hæmorrhage from wounds that penetrate into one of the heart-cavities is larger than that from non-penetrating wounds. Wounds penetrating perpendicularly bleed more than those passing obliquely through the heart-wall. In the latter case, the wound canal is longer and, therefore, a condition more favorable to the coagulation of blood in it exists. Also in an oblique wound canal the wound surfaces are more tightly pressed against each other during the systolic contractions of the part. (See Fig. 2.)

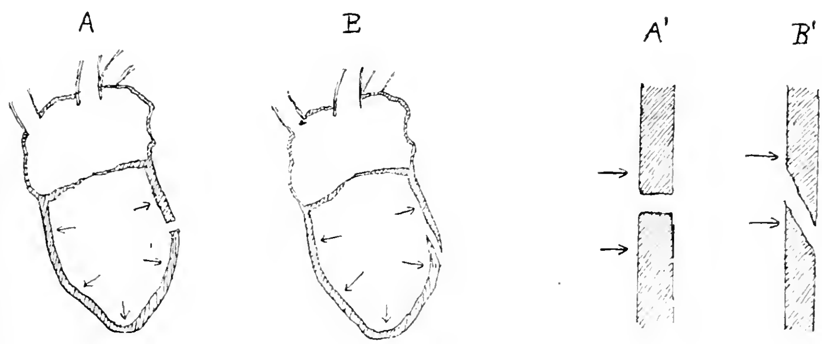

FIG. 2.

To illustrate the Closure of a Wound passing obliquely through the Heart-Wall. (The Arrows represent Lines of Force.)

Wounds of the right ventricle bleed more than do those of equal size made on the left side. Although the pressure of blood in the left ventricle is far greater than in the right one, and, therefore, the tendency to washing away of the clots in the wounds far greater, nevertheless, wounds on the left side are closed by coagulation more rapidly than on the right side. This will be readily understood if we take into account the greater thickness of the wall of the left ventricle and, hence, the greater length of the wound canal. In addition to this, the blood in the right ventricular cavity contains a larger amount of CO_2 and, hence, coagulates more slowly than that in the left. Thus a penetrating wound 2 mm. long could be made

in any part of the left ventricle and the hæmorrhage could be stopped by means of a gauze compress. Such wounds will often close without suture. If the wound in the left ventricle is larger than 2 mm., the animal regularly bleeds to death unless a suture is applied. Wounds 2 to 3 mm. long in the right ventricle of the rabbit will regularly cause fatal hæmorrhage unless the wound is sutured. If the suture be not employed in even the smallest incised wounds made in the auricles, the animal will bleed to death.

The hæmorrhage from all these wounds was most frequently systolic; during each contraction a small stream of blood squirting from the wound. Diastolic bleeding was, however, occasionally observed, especially where the wounds were very large. If the wound is very large (4 or more mm. in the rabbit; 6 to 20 mm. in the dog) and gapes, the blood will simply run out of the wound during the diastole. The same can occur where the opening is of such a size and shape that it closes during each systole and gapes during the diastole of the part. The systolic hæmorrhage is always one under pressure and, hence, greater than the diastolic bleeding.

Series II.

THE NUMBER OF SUTURES THAT MAY BE APPLIED TO THE HEART-MUSCLE WITHOUT INTERFERING WITH ITS FUNCTIONAL ACTIVITY. THE METHOD TO BE FOLLOWED.

The purpose of this series of experiments was to determine the amount of suturing that the heart could stand and the best method of passing the sutures.

In sewing the heart-muscle, the stitches may be passed through a part or through the whole thickness of the cardiac wall. There are some advantages in having the suture pass through a small part of the muscle. In the first place, although the hæmorrhage from the needle puncture is never considerable, except when a blood-vessel is injured, its quantity is proportional to the amount of heart-wall punctured by the needle. When the suture passes through the pericardial layer only there is generally no bleeding at all. In medium-

sized dogs, the visceral pericardium measures about one-half a millimetre in thickness. Unless a large vessel be injured, the passage of a suture through this layer occasions no bleeding at all. The deeper the suture is passed, the larger is the haemorrhage.

In the second place, the pericardium, being the toughest part of the heart-wall, is less apt to tear when little muscular tissue has been included in the suture with it. Should the suture tear through the muscular layer, if a considerable part of the latter is included, it is also very apt to tear out through the pericardium. This accident happened in several of my experiments.

As the size of the animal increases, so also does the force of each contraction of the heart become greater, and hence the greater is the strain that the heart-muscle and the sutures have to withstand. In the dog's heart the sutures that included a considerable part of the thickness of the wall tore out oftener than did those similarly passed through the cardiac muscle of rabbits. In other words the larger the animal, the more apt is a deeply passed suture to tear out. I believe, therefore, that sutures which penetrate through the epicardium and superficial layers of the myocardium only are most certain to hold. The sutures can be passed to this depth with a minimum amount of bleeding and a maximum of certainty that they will hold. Where, however, a very large wound exists and this superficial suture would not suffice to approximate the wound surfaces, a deeper suture or two tiers of suture must be applied.

Another point of technique to which I must call attention is that the sutures should be drawn tight and tied during the diastole of the part operated upon. In the heart of the smaller rabbits this was often impossible on account of the great rapidity of the heart's action. In the larger rabbits and in the dogs, however, where the heart does not beat so rapidly, it is always possible to tie the sutures during a diastolic relaxation. The strain upon both the suture and the heart-muscle is less during both systole and diastole when the suture is tied during diastole than when it is tied during systole. The results I obtained in my experiments with this "diastolic suture" have been very satisfactory. And I may here state that, from the time that I

passed my sutures only through the superficial layers of the heart-wall and tied them only during a diastolic relaxation of the part operated on, I saw very few of my sutures tear through the muscle and so become loose. Suture of the ventricles is easier than that of the auricles, because the former are easier of access and their walls thicker.

In order to discover the maximum amount of suture that the heart could stand, I made a number of interrupted and continuous sutures extending over various distances of the heart-wall. Regarding the advantages of the interrupted over the continuous suture, I shall speak in Series III. The functional activity of the rabbit's heart is not interfered with even by a very extensive line of stitches. In several animals (see Experiments XXIV and XXXIII, pp. 516 and 518), a continuous suture was applied beginning at the base of the right ventricle and extending to the apex and continuing over the wall of the left ventricle to the left auriculo-ventricular sulcus. All the animals operated on in this way recovered and remained well during the full time of observation (3 to 12 weeks). In one animal the suture ran over a part of the auricles also (see Experiment XLI, p. 518). This animal likewise remained well. Of the histological changes in the hearts of these animals I shall speak later. It may be said in this place, however, that the pathological changes consisted mainly in a connective tissue proliferation between and for a small distance around the sutures, while the adjoining muscular fibres preserved their normal appearance.

Series III.

LARGE WOUNDS OF THE HEART IN THE RABBIT AND THEIR SUTURE.

In this series of investigations, I obtained most unexpected results. Small wounds (2 to 5 mm.) of the rabbit's heart when sutured, heal rapidly and well, so that the process of cicatrization of the wound is completed in about 14 days. The interrupted suture has many advantages over the continuous suture. The former, it is true, takes a somewhat longer time in its application than the latter, but it is more certain to hold. If one stitch of a continuous suture should tear out, the whole suture would naturally become loosened, with the most

disastrous results. Besides, the interrupted suture does not compress so many bundles of muscle fibres as does the continuous (see Figs. 3 and 4). My histological investigations have shown me that this is a distinct advantage, because all the muscle fibres that are compressed

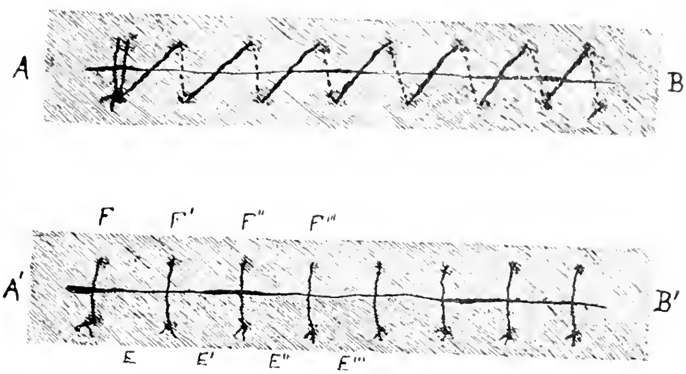

FIG. 3.

The Continuous and the Interrupted Suture. Compare this with Fig. 4.

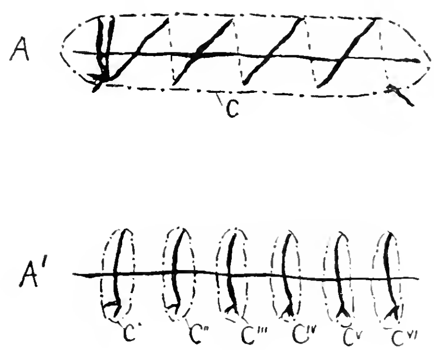

FIG. 4.

The broken line C in A is to show the area within which the muscle fibres are compressed by the continuous suture AB (Fig. 3). A′ represents the condition in the case of the interrupted suture A′ B′ (Fig. 3).

by the ligature or suture eventually atrophy and are replaced by connective tissue.

In very large wounds (4 to 10 mm.) of the heart in rabbits, the hæmorrhage is of course profuse, and unless some method to stop the

bleeding is used and the suture rapidly applied, the animal will bleed to death in a few moments.

FIG. 5.

To illustrate the Size of the Incision made in Exp. XXXVIII. Compare with this Figs. 6 and 7.

FIG. 6.

Cut Surface of Heart Two Months after Exp. XXXVIII. (Light part of figure is the scar tissue.)

FIG. 7.

Section of Heart of Rabbit (Exp. XXXVIII). Incision is at right angle to that in last figure.

I have described, in Series I, the method by means of which a ligature may be tied around a part of the ventricles (p. 492, and Fig. 1). It occurred to me that by means of this expedient, I should be able

to make very large wounds in the ventricles of my animals by a true "bloodless method." Thus in a number of experiments, after the temporary ligature had been applied, a small scalpel was pushed through the whole thickness of the heart, perforating first the left ventricular wall, then the septum, then the right ventricular wall, and then made to cut downward through the heart-muscle until the

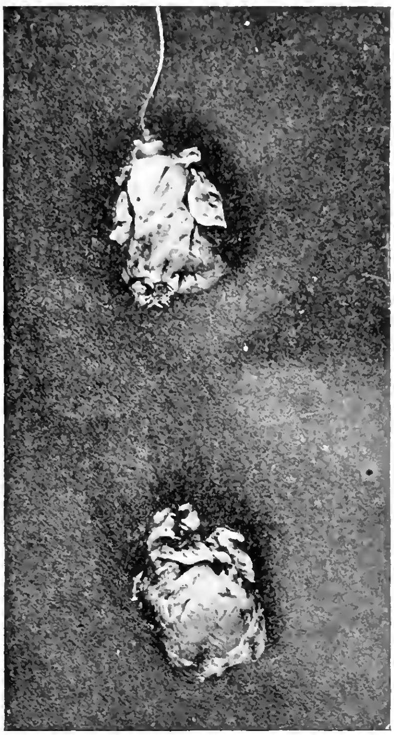

FIG. 8.
Rabbit's Heart, Three Months after the Removal of the Lower Third of the Ventricles. (Experiment XXI.)

knife emerged at the apex of the heart. In this manner, the lower part of the heart was cut into two parts and both ventricular cavities opened. On account of the provisional ligature applied above the highest point of incision there was no hæmorrhage. The whole wound about 2 cm. long, was closed by interrupted sutures of fine silk. The provisional ligature was then removed. Of the 6 ani-

mals thus operated on, one died of hæmorrhage because, during the manipulations, the ligature slipped, a second animal died from sepsis, the remaining four recovered and remained well during the full period of observation (1 to 12 weeks). (Experiments XXVI, XXVII, XXXI, XXXVIII, XXXIX, XLII; see Figs. 5, 6 and 7.)

In a similar manner, the incision was made transversely to the axis of the heart extending over the whole wall of one ventricle. This large gaping wound healed and the animal remained well.

In another series of animals, a tobacco-pouch suture was applied around the heart, so that one-fifth, one-fourth, one-third and even almost one-half of the ventricles was below the ligature. This was then drawn fast and knotted. The part of the heart below the ligature was then cut off and the raw surface covered over by a sero-serosa suture. A number of animals thus operated on remained well (Fig. 8).

In like manner one or both auricular appendages and, with them, parts of the auricles themselves could be tied off and cut away.

And, finally, it must be mentioned that in not a single one of all my experiments did sudden cardiac arrest occur.

PART III.

THE HISTOLOGICAL CHANGES THAT OCCUR IN THE PROCESS OF HEALING OF CARDIAC WOUNDS.

While the literature concerning the histological changes that take place in the healing of wounds of the voluntary muscles is large, that dealing with the healing of wounds of the heart is very small.

Histologically, the cardiac muscle is peculiar in that its fibres contain transverse striations like all voluntary muscles, but that they are not controlled by the will. The muscular fibrillæ have no sarcolemma and the fibres anastomose with each other, so that the "whole heart may be regarded as a single branching muscle fibre, whose branches anastomose with each other."

It seems to be fairly certain that in the healing of wounds of the voluntary muscles, a regeneration of the muscle fibres is to some extent possible. A large number of these young muscle fibres

eventually atrophy and are replaced by connective tissue, but some of them remain as regular muscle fibres. Authorities differ as to the origin of these new fibres. While Waldeyer,* Weber,† Hofmann,‡ Robert,§ Zenker‖ and others declare that the new fibres are derived from the spindle cells in the interfibrillar connective tissue, other investigators (Renaud,¶ Virchow, Duplaix,** etc.) believe that the new fibres are derived from the old ones.

The question whether a regeneration of the muscle fibres of the heart ever takes place, is one concerning which there is also great difference of opinion. Heschl†† believes that he has frequently seen new muscle fibres in microscopic specimens from hypertrophic human hearts. Goldenberg‡‡ declares that it is possible that in hypertrophy of the heart "new muscle fibres are formed by a longitudinal division of the old fibres." Tangl,§§ on the contrary, denies that a regeneration of the heart-muscle fibres occurs. He never found cells which he would acknowledge to be transitional forms between a connective-tissue cell and a muscle-cell, and never saw an undoubted mitotic division of the nucleus of a muscle fibre.

Bonome‖‖ and Berent¶¶ investigated this subject experimentally on the rabbit's heart: their conclusions on this point agree with those of Tangl.

The method in my examinations was the following: Each animal was killed by a sharp blow on the back of its neck, the heart was quickly removed and opened, and the tissues fixed by immersion for 24 hours in a 10 per cent solution of formalin in water or in Müller's fluid. The specimens were hardened in alcohol of increasing con-

* Waldeyer, Virchow's *Archiv*, xxxiv, 473.
† Weber, ibid., xxxix, 210.
‡ Hofmann, ibid., cl, 161.
§ Robert, Ziegler's *Beitrage*, x, 109.
‖ Zenker, *Verhandl. d. X. Internat. Congress.*, 1890, ii.
¶ Renaud, *Gaz. méd. de Paris*, 1877, p. 361.
** Duplaix, *Archives de med. gén.*, 1885.
†† Heschl, *Comp. d. path. Anat.*, p. 176.
‡‡ Goldenberg, Virchow's *Archiv*, ciii, 88.
§§ Tangl, ibid., cxvi, 432.
‖‖ Bonome, Ziegler's *Beitrage*, 1889. v, 267.
¶¶ Berent, Inaug.-Dissert., 1887.

centration, embedded in celloidin, sections cut, and then stained with hæmatoxylin (Delafield), Van Gieson's fluid, Bismarck brown, carmine, and Weigert's fibrin stain.

In the normal heart-muscle of the rabbit the longitudinal is much more marked than the transverse striation. The nuclei are long with rounded ends and generally stain strongly with the ordinary staining dyes.

Within the first 24 hours the whole wound area is infiltrated with red and white blood cells; there is fibrin between the edges of the

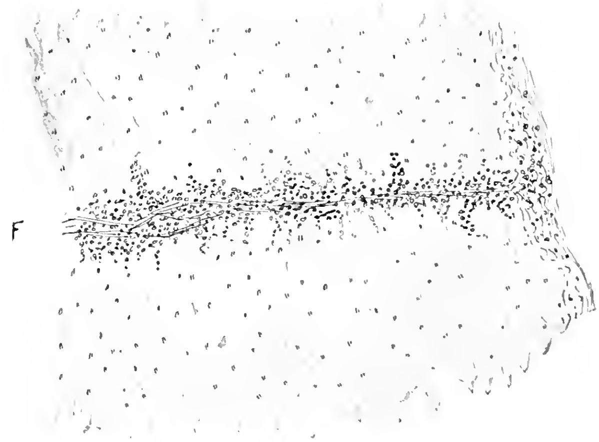

FIG. 9.

From Wound of Rabbit's Heart 24 hours after operation. Fibrin between the lips of the wound; beginning round-cell infiltration. X60.

wound and between the neighboring muscle fibres (see Fig. 9). The interfibrillar capillaries are distended with blood. In a few instances a well-marked "fragmentation" of the muscle fibres could be seen.

After 48 hours the wound area is so thickly infiltrated with leucocytes that it is often difficult to find the exact location of the wound. The fibres that are compressed by the sutures are undergoing degeneration, as is shown by their granular aspect and by the fact that their nuclei will often not take any stain. Around the sutures the infiltration of small round cells is very large. The nuclei of the muscle cells near the wound seem to be increased in number and

some seem to be undergoing amitotic division. In the degenerated muscle fibres, the protoplasm is broken up and collected in little masses. Among these fibres I have occasionally seen nuclei undergoing karyokinetic changes. I have never, however, been able to feel convinced that these nuclei did not belong to the interfibrillar stroma, or, perhaps, to the capillary wall and not to the muscle fibre.

By the fourth day the area of operation is still thickly infiltrated with small round cells. The degenerated muscle fibres have begun to disappear. Numerous young spindle cells can now be found. Their shape and size vary considerably, and they are very frequently

FIG. 10.

To illustrate the Growth of the young Spindle Cells between the muscle fibres. From the heart of the rabbit eight days after operation. X60.

encountered in and just under the pericardium and around the sutures. The nuclei in some of these cells are undergoing mitotic division. Around the sutures just under the pericardium are to be found some large giant cells with numerous nuclei.

On the 6th or 7th day the whole area of the wound is filled by these spindle cells; they have replaced all the degenerated muscle fibres and have even penetrated in places between the uninjured ones.

By the 10th day the granulation tissue is beginning to change into fibrous tissue, the spindle cells grow longer and thinner, and the small round cells are disappearing (Fig. 10). Giant cells still occur

in the neighborhood of the sutures. This new-formed connective tissue is much larger in amount under the pericardium than near the endocardium, so that one has the impression of its being derived for the most part from the subepicardial connective tissue.

At the expiration of four weeks, the whole wound is filled by the connective tissue, which extends for various distances into and between normal muscle fibres. This connective tissue is especially prominent, as stated above, between and around the sutures. In the heart where,

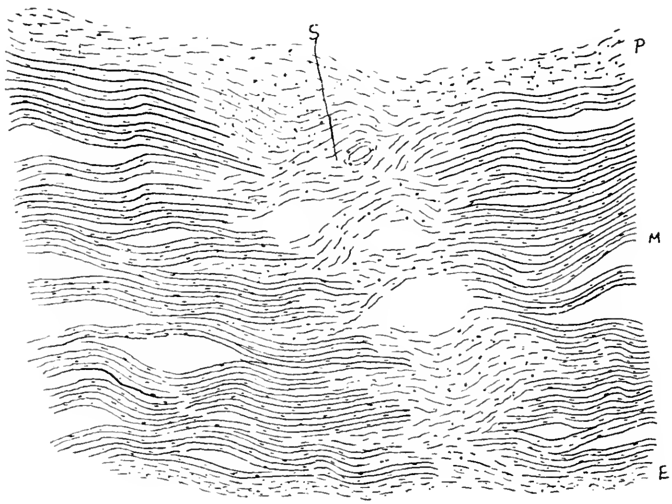

FIG. 11.

Cross-Section of perforating Wound of Rabbit's Heart three weeks after operation. P, Epicardium; M, Myocardium; E, Endocardium; F, Fibrous Tissue of Scar. (Semi-diagrammatic.) X40.

without previous injury to the organ, a continuous suture has been passed, the whole area of the line of sutures is transformed into connective tissue, so that there is a band of fibrous tissue running along the heart-wall (Fig. 11).

In the hearts in which an interrupted suture has been made, the condition is entirely different. In and around each suture there is considerable connective tissue formation, but between each of two adjacent sutures, some normal muscle fibres remain. Thus in Fig. 3

transverse section through any part of the line of suture AB would show connective tissue bands, while transverse section through any of spaces, E, E′, E″, E‴, etc. (Fig. 3, A′B′), would show that in this situation the muscle fibres have been preserved. Near and around the sutures, F, F′, F″, etc., the connective tissue would have replaced the muscle tissue.

CONCLUSIONS.

It would, of course, be incorrect to attempt to draw conclusions as to the dangers and the chances of success of suture of cardiac wounds in man from the results obtained by animal experimentation. Animals are placed in very unfavorable conditions after the operation. They are very restless and cannot be kept quiet. Ideal cleanliness is impossible and the animals may infect their wound by rubbing the external wound against the dirt on the floor of their cage. From the animal mortality in these investigations no rigid inferences applicable to human beings can therefore be made.

Some conclusions of importance can, however, be drawn. Above all, my experiments seem to show that the mammalian heart will bear a much greater amount of manipulation than has hitherto been suspected. Very large wounds of the heart can heal and the healing process occurs in a manner entirely analogous to that in other muscular tissues. Even an extensive suture of the heart-wall of rabbits and dogs, although we know that thereby a large number of muscle fibres are destroyed and replaced by connective tissue, does not interfere with the function of the cardiac muscle as a whole.

Can some of the results in the above recorded experiments be, with some restrictions of course, applied to the human heart? I think that this question must be answered in the affirmative. If we compare the knowledge we possess of wounds of the heart in man, with that obtained from animal experiments, and find that they agree in all essential particulars, then we are justified in reasoning by analogy that suture of wounds of the heart in man will give results similar to those obtained in the animal. In the last few decades, the advances made in all the branches of medicine—especially in pathology, bacteriology

and surgery—have been due to a great extent to the generalization of the results of animal experimentation. To the careful and critical investigator, the results obtained in the animal experiment have always been of the greatest value in indicating to him the possibility of results to be obtained by similar procedures in the human body.

From the study of wounds of the heart in man, and from the results obtained in my experiments, this conclusion seems therefore justified: wounds of the heart in man, when all other means have been tried and found wanting, can and ought to be closed by suture. The application itself of the suture is devoid of the one great danger that was feared in the past, *i. e.* of sudden arrest of the heart during the manipulations incident upon the application of the sutures. The number of sutures should be as small as possible so as to limit the amount of connective tissue which will be formed; for all the muscle fibres that are compressed by the sutures eventually atrophy and are replaced by new-formed connective tissue. It is probable that this connective tissue will not lead to degenerative changes in the heart-muscle. On the post-mortem table, fibrous plaques are often found in the otherwise normal human heart. In a number of the muscles of the body fibrous bands—tendinous intersections as they are called—are normally found. In the large number of microscopic sections of the heart-muscle that I have examined, I could find no evidence of pathological changes in the muscle fibres some distance from the scar. For similar reasons the suture should always be an interrupted one. We have shown that there are dangers and disadvantages in the continuous suture both on theoretical grounds and in practical use.

The sutures should be passed through as little of the heart substance as possible; if they penetrate the epicardium and a small part of the thickness of the heart-muscle it will generally be sufficient.

When the heart's action is not too rapid, each suture should be tied during a diastolic relaxation of the part under treatment. On this point we have not yet any experience in man. Cappelen,* in his patient, tied the sutures during systole. Rehn† tied them in his case during diastole. Only time and further experience will show

* Cappelen, loc. cit.

† Rehn, loc. cit.

how much importance is to be attached to this point. All that can be said, in the present state of our knowledge, is, that on theoretical grounds and from animal experimentation, it must be considered safest to tie the sutures during diastole.

On first sight, it might appear difficult to apply sutures to an organ in such constant motion as is the heart. In practice, however, the difficulties have been proven not to be so great as might appear.

The heart may be grasped with a forceps and the needle and suture easily passed. It is no more difficult to pass and tie a suture in a large dog than in a small rabbit. Hence we should infer that the difficulties of this procedure in the human heart, are not so great, a fact that has been borne out by the experience of those surgeons who have reported cases of heart-suture in man.

The cases will always be few in which this extreme method of treatment—for so we must style it—is necessary. Indeed, of the patients that come under the care of the surgeon, there are some who will recover from even large heart wounds without any local treatment at all. Cases have been recently reported by Conner, Brugnoli, Hamilton and others, where after wounds as large as three centimetres, the hæmorrhage ceased spontaneously and the patients recovered. One cannot say, therefore, that wounds larger than a certain size must always be sutured. Each case must be carefully considered by itself.

When we examine the nine cases of suture of the human heart in man (see pages 487 to 490) we cannot but hope for considerable success from this new method of surgical procedure. Of the nine cases, four recovered entirely, and four died of complications referable to other organs—quite an encouraging record in a few cases.

Finally, I may be permitted to summarize these conclusions as follows:

1. Suture of a wound of the heart as a final resort is an operation worthy of consideration in some cases and often justifiable.

2. Suture of wounds of the heart in animals, and also in man, is devoid of the danger of sudden arrest of the heart, due to the manipulation of the heart incident to the procedure, unless Kronecker's coördination centre be injured.

3. The suture should be an interrupted one of silk, applied in most cases so that the epicardium and superficial layers of the myocardium should be the only ones penetrated, and tied, when possible, during diastole.

4. No stated indications can be given as to the cases that are operable or the time when the operation should be done. Each case must be considered by itself for symptoms which would justify operative interference.

In conclusion, I desire to take this occasion to state that through the kindness of Dr. J. Mikulicz, Professor of Surgery in the University of Breslau, the laboratory of the Surgical Clinic was placed at my disposal, and that the greater number of my experiments were made in that laboratory.

PROTOCOLS OF EXPERIMENTS.

I. 14-vi-'98. Rabbit. Weight 1480 gm.; resp. 132; pulse 240. Ether narcosis. Median incision 4 cm. long. Pectoralis major cut near its insertion and reflected outwards. Cartilages of 4th and 5th ribs cut close to the left border of the sternum and also turned back, together with the muscles attached to them. Triangularis sterni cut. Internal mammary artery drawn outwards. Pleura intact. Bleeding small. Pericardium exposed on its whole anterior surface. Heart's action regular. It is uninfluenced by pressure on the heart with a blunt instrument through the pericardial sac. Pericardial sac now sewn to the thoracic muscles around the edges of the wound, and then opened by a longitudinal incision. Heart's action still regular. Light pressure on heart with blunt forceps has no appreciable influence on heart's action. When firmer pressure is made, the heart lags behind for a moment, and then beats very rapidly. The action of the heart seems to be unaffected by pressure on the auricles. With a small teasing needle the left ventricular wall is punctured. The next systole is now delayed for a moment, then follows a short period of irregular contractions. The needle is then made to penetrate the cardiac muscle deeper and deeper. As the needle penetrates the endocardium the same arhythmia is observed. The needle is now withdrawn. There is some hæmorrhage for two minutes. The heart is now punctured several times in several parts of the right and left ventricles, the needle penetrating each time into the cavity of the heart. The bleeding from all these punctures is small and soon ceases. Toilette of the pericardial sac. Ribs bent back into place. The cut edge of the left pectoralis major is now sewn to the insertion of the right pectoral muscle. Skin closed by continuous silk suture. Iodoform collodium dressing. After the operation animal is weak on its front legs, probably due to the manner in which it was tied down. Heart's action, as heard with stethoscope, weak but regular. Loud pericardial friction sound. R. 120; pulse 260.

After one-half hour animal is running around and eats grass.

15-vi-'98. Animal seems to feel well; it runs around as if nothing had been done.

17-vi-'98. Pericardial friction sound has disappeared. Heart's action regular, 200 to the minute. Resp. 68.

19-vi-'98. Skin wound healed. Animal well.

26-vi-'98. Animal seems to be well in every respect.

27-vi-'98. Killed. Post-mortem examination: Skin wound clean and healed; pericardial sac closed, adherent to heart over its greater part; small abscess under fifth left rib. On the surface of the heart, the points of puncture can be seen as small depressed spots.

II. 15-vi-'98. Rabbit. Weight 1550 gm.; pulse 180; resp. 80. Ether narcosis.

Incision as in Experiment I. 3rd, 4th and 5th cartilages cut close to left border of sternum and reflected. Internal mammary artery was torn but caught and tied without much loss of blood. Pericardial sac sewn to muscles in usual manner and opened as in last experiment. Heart is now firmly grasped with forceps and pulled out of the wound. Delay in next systole, followed by irregular heart's action for one minute; then regular but rapid contractions. Animal is now made to inhale three drops of chloroform. Heart's action becomes very slow, about 60 to the minute, then more rapid as the chloroform is taken away. With a small scalpel, the left ventricular wall is punctured during its diastole (inhalation of chloroform). Immediately there is considerable hæmorrhage, the blood squirting about 30 to 40 centimetres high at each systole. There is no diastolic loss of blood. While the scalpel penetrated the pericardium and likewise the endocardium, marked irregularity of heart's action. Wound tamponed with gauze; after about three minutes, tampon removed and hæmorrhage has ceased. Animal is again allowed to inhale a few drops of chloroform. Heart's action at once becomes very slow and after a few beats ceases altogether. Chloroform is at once removed. Animal cries out and has a general convulsion. Heart can be seen to be filled by dark blood. Heart again begins to beat, slowly at first, then more rapidly, and at same time wound begins to bleed again. Hæmorrhage again ceases after tamponade. Thorax wound then closed in the usual manner. Duration of operation 38 minutes. After two hours, R. 60; P. 200. Animal is eating grass.

17-vi-'98. Animal appears to be well. Skin wound firmly closed.

19-vi-'98. R. 68.

26-vi-'98. Animal perfectly well.

27-vi-'98. Killed by blow on back of neck. Post-mortem examination shows that pericardial sac is closed and adherent to thoracic wall in front. Parietal pericardium adherent to the visceral layer only at point of wound in left ventricular wall, where both seem much thickened. Pleura intact. Cardiac wound firmly closed.

III. 15-vi-'98. Rabbit. 1400 gm.; R. 68; P. 260. Ether. Operation in every respect like Experiment II, except that wound in left ventricle is made during the systole of the part. Hæmorrhage is much larger than in preceding experiment. It is systolic in character but there seems to be also

some slight diastolic oozing of blood. Same irregularity of heart's action as in previous experiments. Wound, which is 3 mm. long, in transverse axis of heart, does not gape. Pressure applied to it by means of tampon of gauze. Hæmorrhage ceases but begins anew as soon as the gauze is removed. Bleeding is very large; heart's action becomes slower and irregular, hæmorrhage less; heart finally stops in diastole. Post-mortem examination: Wound 4 mm. long in left ventricle, heart chambers almost empty, marked anæmia, of all the abdominal organs.

IV. 15-vi-'98. Rabbit. 1550 gm.; R. 68; P. 285. Ether. Usual incision; 4th, 5th and 6th cartilages reflected. Left pleural cavity opened, but at once closed with gauze tampon. Pericardial sac sewn to muscles of thorax and opened. During the inhalation of a few drops of chloroform, an incision of 3 to 4 mm. in length is made in the wall of the left ventricle during a systole. Strong systolic bleeding. Heart is at once drawn forwards and wound closed by means of pressure with anatomical forceps. Five interrupted sutures applied. These penetrate only the pericardium and superficial layers of myocardium. Bleeding from needle punctures is small and ceases as soon as suture is tied. Heart's action irregular while wound is made and sutured. Thorax wound then closed in the usual manner. It is to be noted that the compression of the edges of the wound in the heart with the forceps, had no appreciable influence on the character of the heart's action.

17-vi-'98. Animal eats and seems well.

19-vi-'98. Skin wound healed.

29-vi'-98. Wound healed. Killed in usual manner.

Post-mortem examination: Parietal pericardium adherent to visceral layer over large part of surface of heart and considerably thickened over situation of wound in heart muscle. Wound in ventricular wall firmly cicatrized and the scar seems somewhat depressed.

V. 17-vi-'98. Rabbit. 1940 gm.; R. 60; P. 280. Ether. Pericardial sac exposed and opened in the usual manner. During a diastole of the ventricle, incision 3 mm. long in middle of left wall, and not perforating into heart cavity. Heart's action remains regular. Hæmorrhage is large and systolic. Bleeding wound clamped by means of artery forceps. Forceps tear through muscle and bleeding again ensues. Wound again clamped. After 3 minutes clamp is loosened and removed and bleeding has ceased. Wound is seen to be lacerated one, measuring about 6 mm. in its longest diameter. Animal seems very weak. In this experiment, while the chloroform was given, the animal seemed to have dyspnœa and during the deep respiratory movements, heart was partly forced out of wound of thorax and hæmorrhage was greater than at any other time.

19-vi-'98. Animal jumps around and eats grass.

24-vi-'98. Animal has just given birth to five fœtuses of about 3 weeks of age.

26-vi-'98. R. 80. External wound healed.

10-vii-'98. Well in every respect.

30-vii-'98. Is gaining weight rapidly.

15-viii-'98. Well.

8-ix-'98. Animal perfectly well.

VI, VII, VIII. Three rabbits weighing 1480, 1720, 1900 gm. respectively. Ether anæsthesia.

In these three animals, the pericardium was exposed but not opened. By means of a needle, the heart muscle was injured by punctures through the pericardial sac and the heart observed. In all three animals, it was noted that—no matter where the wound was made—as the pericardial sac became more and more filled by blood, the heart was pressed more and more firmly against the anterior wall of the pericardial sac and could be plainly seen through it. Heart's action gradually became irregular and slower in each experiment. In VI, heart's action ceased in six minutes; in VII in fourteen minutes; in VIII, in sixteen minutes. The post-mortem conditions in the three animals were almost identical. In each the apex of the heart was found firmly pressed against the anterior wall of the pericardial sac; the heart cavities of each animal were almost empty.

IX. 17-vi-'98. Rabbit. 1590 gm.; P. 148; R. 48. Ether. Internal mammary artery injured and secured only after considerable hæmorrhage. During this procedure, a large rent was made in the left pleura. Thoracic wound at once closed. Animal remained very weak for several days after the experiment.

28-vi.-'98. Killed by blow on neck. Large abscess in left pleural cavity.

X. 17-vi-'98. Rabbit, medium size. R. 48; P. 208. Ether; incision and resection of 4th and 5th ribs in usual manner. Heart is drawn upwards by means of a forceps and during diastole, a wound 2 mm. long is made obliquely in middle of right ventricle. Characteristic arhythmia for three minutes. Hæmorrhage systolic. Heart is allowed to drop back into pericardial sac and wound is compressed with gauze. After several minutes bleeding stops. External wound closed in usual manner after toilette of pericardial sac. Respiration 60; pulse 180. After one hour animal jumps around in its cage as if nothing had been done to it.

23-vi-'98. Skin wound healed.

26-vi-'98. Well in every respect.

28-vi-'98. Killed. Skin wound clean and firmly healed. Pericardium adherent to heart only over the spot where the right ventricle had been wounded. Other organs normal.

XI. 19-vi-'98. Rabbit. 1870 gm., R. 88; P. 224. Ether. Heart, exposed in usual manner, drawn into wound and firmly compressed with anatomical forceps; heart now beats very irregularly. It is then punctured with a scalpel, the blade passing through the wall of the left ventricle, septum and wall of right ventricle.

Enormous systolic hæmorrhage, and some oozing during each diastole. Pericardial sac packed with gauze. After three minutes gauze is removed; hæmorrhage has ceased. External wound closed in the usual manner. Animal is very weak. R. 92; P. 240. Died three hours later. Through an error, the body of the animal was removed before a post-mortem examination had been made.

XII. 19-vi-'98. Rabbit. 1200 gm.; R. 68; P. 240. Under ether, heart is exposed in usual manner. Right auricle punctured at its middle with a "teasing-needle." As the needle is withdrawn, a rent is made in the auricular wall, and a large hæmorrhage ensues. Wound is at once tamponed with gauze. After 4 to 5 minutes tampon is removed; bleeding has stopped. External wound quickly closed. Animal is found dead in its cage the next morning. Post-mortem examination: Pericardial sac filled with clotted blood.

XIII. 20-vi-'98. Rabbit. 1480 gm.; R. 52; P. 192. Ether. Heart, exposed in usual manner, is drawn forwards with forceps, and seven interrupted sutures of fine silk passed through wall of left ventricle. The sutures are 2 mm. apart and do not penetrate into the heart cavity. During the passage of the needle, characteristic arhythmia. Thorax wound closed.

26-vi-'98. Skin wound healed.

10-vii.-'98. Small abscess in line of wound opened. It does not communicate with thoracic cavity.

20-vii-'98. Abscess cavity clean and healed.

22-vii-'98. Killed. There is a small abscess of deeper layers of wall of thorax, extending into the mediastinum but not into the pericardial sac. Parietal pericardium adherent to the visceral layer of greater part of anterior surface of heart. On account of thickened pericardium, it is almost impossible to see the exact line where the sutures had been applied.

XIV. 20-vi-'98. Rabbit. Weight 1400 gm.; R. 52; P. 248. Continuous suture of seven stitches in upper part of right ventricle. Characteristic arhythmia. During the toilette of the pericardial sac, animal awakes from the anæsthesia, struggles violently, and both pleuræ tear. Heart's action at once becomes very slow, and stops after four beats.

XV. 22-vi-'98. Rabbit. 1840 gm.; R. 56; P. 124. Ether. Heart exposed in usual manner. Heart is drawn forwards with mouse-tooth forceps and one fine silk suture is inserted in the lower part of the wall of the left ventricle. By means of this thread, heart is now drawn well forward. When lower part of ventricles is compressed with forceps, characteristic arhythmia is observed. Through the pressure of the mouse-tooth forceps, the cardiac muscle is injured over the lower part of the septum ventriculorum, and the left (?) ventricular cavity is opened. A tremendous systolic hæmorrhage follows. Wound is at once closed by three interrupted sutures. Toilette of pericardial sac. External wound closed.

23-vi-'98. Animal jumps around and eats grass.

10-vii-'98. Well in every respect.

30-vii-'98. Is gaining in weight, now weighs 1920 gm.

10-ix-'98. Is perfectly well.

XVI. 22-vi-'98. Rabbit. 1715 gm.; R. 48; P. 140. Ether. Four interrupted sutures through upper part of wall of right ventricle, 2 mm. apart, and all penetrating into ventricular cavity. Bleeding from needle-punctures is small. Toilette of pericardial sac. Thoracic wound closed in usual manner.

10-vii-'98. External wound healed and animal gaining in weight.

2-viii-'98. Killed. Post-mortem examination: External wound healed. Small abscess under skin containing about one drop of pus. Parietal pericardium adherent to the visceral layer only over the situation of the sutures. Surface of heart has smooth normal appearance.

XVII. 23-vi-'98. Rabbit. 1000 gm.; R. 68; P. 184. Ether. Six interrupted silk sutures in upper part of wall of left ventricle. Suture includes only pericardium and superficial layers of myocardium. There is almost no bleeding. Characteristic arhythmia absent.

10-vii-'98. Wound healed.

2-viii-'98. Animal has increased in weight, now weighs 1400 gm.

3-viii-'98. Killed. Post-mortem: External wound clean and healed. Pericardial sac closed and adherent to visceral pericardium of greater part of anterior surface of heart. Heart muscle, on section, looks normal.

XVIII. 23-vi-'98. Rabbit. 1920 gm.; R. 100; P. 220. Ether. Wound 3 mm. long and 3 mm. deep made over the middle of the septum of the ventricles on the anterior surface of the heart. Hæmorrhage systolic. Wound closed by five fine silk sutures. External wound closed in usual manner.

26-vi-'98. Animal seems well.

1-vii-'98. Is found dead in its cage. Post-mortem examination shows large abscess in thoracic wall. Wound in heart-wall firmly closed.

XIX. 24-vi-'98. Rabbit. 1580 gm.; R. 76; P. 200. Ether. Usual operation for exposure of heart. During this procedure, the internal mammary artery was cut; it was however ligated before much bleeding had occurred. One suture in the middle of the wall of the left and another in the middle of the wall of the right ventricle. Continuous suture of six stitches in the lower part of the wall of the right auricle. All the sutures penetrate into the heart cavities. The bleeding from the needle-punctures is small and is immediately arrested when the suture is drawn tight and tied. Heart's action rapid and irregular.

26-vi-'98. R. 92. Animal appears to be well.

10-vii-'98. R. 92. External wound healed.

22-vii-'98. Killed. Wound in auricle firmly healed and parietal pericardium adherent to it. All other organs normal.

XX. 24-vi-'98. Rabbit. 1380 gm.; R. 88; P. 200. Ether. Penetrating wound, 3 mm. long over septum of the ventricles. Hæmorrhage systolic. Four interrupted sutures. A second wound, 4 mm. long, is made in apex of the heart, penetrating into the left ventricle. It is closed by four interrupted silk sutures. Heart's action rapid but strong.

26-vi-'98. Is well. R. 92.

10-vii-'98. Skin wound firmly healed. R. 80.

20-viii-'98. Animal is gaining in weight. Now weighs 1500 gm. Killed. Wounds in heart-muscle are firmly cicatrized and the parietal pericardium firmly adherent to visceral layer over them.

XXI. 24-vi-'98. Rabbit. 1600 gm.; R. 68; P. 200. Ether. Heart exposed in usual manner. A tobacco-pouch suture is applied transversely around the ventricles, so that the lower third of the ventricles is below the suture. This is then drawn tight and tied. The part of the ventricles that is thus cut off from the rest of the heart continues to contract, but one has the impression that each contraction lags behind a moment. The action of the whole heart remains good. The portion of the ventricles below the suture is then excised and the pericardium united over the cut surface. Heart's action remains good and there is no bleeding. Toilette of the pericardial sac. External wound closed in usual manner.

25-vi-'98. Animal seems to be well.

30-vi-'98. External wound healed.

10-viii-'98. Now weighs 1800 gm.

10-ix-'98. Is perfectly well.

20-ix-'98. Killed.

XXII. 25-vi-'98. Rabbit. 1410 gm.; R. 100; P. 280. Ether. A penetrating wound, one centimetre long, is made in the wall of the right ventricle during a diastole. A tremendous hæmorrhage follows which cannot be totally controlled by compression of lips of wound with artery forceps. Five interrupted sutures of silk close the wound and control the bleeding. Several of these sutures, having been tied during systole, tear out through the muscle. Fresh sutures are passed and these are tied during diastole. Every one of these holds.

26-vi-'98. Very weak and will not eat.

27-vi-'98. Found dead in its cage. Post-mortem examination: Pericardial sac filled with clotted blood. The lower two sutures in heart-muscle, having been applied and tied during a systole, have torn through the muscle and the animal has gradually bled to death.

XXIII. 25-vi-'98. Rabbit. 1510 gm. Ether. Incision one-half centimetre long in wall of right ventricle near septum. Closed by continuous suture, each stitch of which is drawn tight during a systolic contraction of the ventricles. Several of the stitches tear through the heart-muscle and enormous hæmorrhage ensues. Heart's action becomes very slow and irregular and soon stops.

XXIV. 27-vi-'98. Rabbit. 1750 gm.; R. 48; P. 220. Ether anæsthesia. A continuous suture was applied to the wall of the left ventricle, beginning at the base and extending to the apex of the ventricle, and the suture is there continued along the wall of the right ventricle from apex to base. Sixteen stitches passed, some of which penetrate into heart cavity, others pass through the pericardium and superficial layers of myocardium only. The bleeding from the superficial needle punctures is much smaller than that from the penetrating punctures. Characteristic arhythmia each time the needle is passed. Several of the stitches tear out of the muscle and have to be applied a second time.

28-vi-'98. Animal appears to be well.

10-vii-'98. Animal has been perfectly well. Skin wound firmly healed.

10-ix-'98. Animal has been steadily gaining in weight. Killed by blow on neck. Post-mortem examination shows that heart's surface is smooth and free, except along line of suture where visceral pericardium is considerably thickened.

XXV. 27-vi-'98. Rabbit. 1570 gm.; R. 61; P. 204. Ether anaesthesia. Right and left appendix auriculæ tied off and cut off. Toilette of pericardial sac.

28-vi-'98. R. 64; P. 220.

10-vii-'98. Seems perfectly well.

29-vii-'98. Animal looks sick.

30-vii-'98. Died. At the post-mortem examination the heart wounds cannot be found, the pericardium is very much thickened over the whole heart. Macroscopically and microscopically heart-muscle is normal. Marked hepatic coccidiosis.

XXVI. 27-vi-'98. Rabbit. 1840 gm.; R. 64; P. 200. Ether. A temporary ligature tied transversely around ventricles so as to include about one-third of the ventricles below the ligature. A small scalpel is then made to pass through the whole thickness of the heart below the ligatures, thus penetrating the wall of left ventricle, the septum between the ventricles and the wall of the right ventricle. The scalpel is then made to cut downwards toward the apex and through it, so that this lower part of the heart is divided into two and both ventricular cavities opened. On account of the provisional ligature, there is no bleeding. The wound, about one centimetre in length is closed by eight interrupted sutures. Provisional ligature then removed. There is no bleeding. Toilette of pericardial sac. Suture of external wound.

10-vii-'98. External wound healed. Animal is well in all respects.

20-ix-'98. Now weighs 2020 gm. Killed. Post-mortem examination shows that heart wound is firmly healed and parietal pericardium is adherent over it.

XXVII. 27-vi-'98. Rabbit. 2000 gm. Same operation as in Experiment XXVI.

10-vii-'98. External wound healed. Animal well.

20-vii-'98. Is losing in weight and looks badly.

29-vii-'98. Found dead in its cage. Large abscess in mediastinum. It does not communicate with heart wound, which latter is firmly cicatrized.

XXVIII. 30-vi-'98. Rabbit. 2160 gm. Same operation as Experiment XXI. After operation animal was very weak and died forty hours later.

XXIX. 30-vi-'98. Rabbit. 2440 gm.; R. 64; P. 220. Ether. Wound 6 mm. long, penetrating left ventricle. Systolic hæmorrhage. Wound closed rapidly by four interrupted sutures. Another wound 3 mm. long and penetrating into cavity of right ventricle made and closed with two sutures. Right appendix auriculæ tied off and cut off below ligature.

8-vii-'98. External wound healed. Animal well.

5-viii-'98. Is gaining in weight.

6-ix-'98. Is very well. To-day gave birth to four young ones.

XXX. Rabbit. 1450 gm. Same operation as in Experiment XXI except that somewhat more than one-third of ventricles was excised. Animal very weak and died eighteen hours after the experiment.

XXXI. 7-vii-'98. Rabbit. 2050 gm. Same operation as in Experiment XXVI. Eight sutures applied superficially in the usual manner. There is no bleeding, and heart's action remains strong and good.

21-vii-'98. Animal has remained well. Killed. Heart wound healed. Scar is broad and thin and bulges a little.

XXXII. 8-vii-'98. Rabbit. 2230 gm. Operation in all respects similar to Experiment XXIV. During the manipulations several of the stitches tore out and animal lost considerable blood. It remained very weak and died ten hours later.

XXXIII. 8-vii-'98. Rabbit. 2450 gm.; R. 64; P. 220. Operation as in Experiment XXIV, except that the continuous suture consisted of 24 stitches.

10-viii-'98. Is well; external wound healed.

10-ix-'98. Well and gaining in weight.

XXXIV. 11-vii-'98. Rabbit. 1850 gm. The same method of operation as in Experiment XXI except that only one-fourth of the ventricles is excised. This animal remained well and at the expiration of seven days, when the skin wound was healed, it was killed and the heart removed for microscopical examination.

XXXV. 12-vii-'98. Rabbit. 1300 gm. Operation similar to Experiment XXI.

10-ix-'98. Animal has remained well and is gaining in weight.

XXXVI. 13-vii-'98. Rabbit. 1200 gm. Ether. Appendix auriculæ and about one-fourth of the left auricle tied off by a ligature thrown around it and cut off.

10-ix-'98. Animal has remained well.

XXXVII. 13-vii-'98. Rabbit. 1200 gm. Operation as in Experiment XXI. Killed after four days for microscopical study of heart.

XXXVIII and XXXIX. 18-vii-'98. Weight of each rabbit 1100 grammes. Each operation as in Experiment XXVI with similar observations made as in that experiment.

10-ix-'98. Both animals have remained well and have gained in weight.

XL. 20-vii-'98. Rabbit. 2000 gm. Operation as in Experiment XXI.

20-viii-'98. Is well and gaining in weight.

XLI. 20-vii-'98. Rabbit. 1500 gm. Same operation as in XXIV except that a part of both auricles was included in the continuous suture.

15-viii-'98. Skin wound healed and animal seems to be well.

20-ix-'98. Killed. Heart surface smooth. No adhesions between parietal and visceral layers of pericardium.

XLII. 20-vii-'98. Rabbit. 1850 gm. Operation as in Experiment XXVI. The provisional ligature slipped, an enormous hæmorrhage ensued, which caused the death of the animal in a few moments.

XLIII to XLIX. In these experiments, wounds as in XXI, XXIV and XXVI were made and the animals killed in one to seven days for histological purposes.

L. Medium-sized dog. Morphine and ether. During the manipulations incident on exposure of the pericardial sac, but pleuræ were opened and before artificial respiration could be begun, the heart stopped beating.

LI. Medium-sized dog. Morphine, gr. ¼. Ether anæsthesia. Tracheotomy performed and cannula inserted into trachea and made ready for connection with apparatus for artificial respiration. Fourth, fifth and sixth ribs exposed by longitudinal incision along left border of sternum. About 1½ inches of each of these ribs excised. Left pleura incised and pericardial sac quickly grasped and drawn up into wound. It was then sutured to the muscles around the edges of the thoracic wound and opened by a longitudinal incision. The heart was then manipulated in a manner similar to that described for the rabbit's heart. It was grasped with a forceps, punctured with needles, a ligature thrown around the lower portion of the ventricles and tied tightly, etc. The results obtained were analogous to those in rabbits. The same arhythmia of the heart, the same irritability of the pericardium and endocardium was present. Hæmorrhage from the needle punctures was *less* than in the rabbits. When the pericardium alone was penetrated, there was no bleeding at all. When the needle was made to penetrate into the ventricular cavity, the hæmorrhage was somewhat larger, but ceased quickly. The right ventricular wall was punctured with the needle; the bleeding was larger than on the left side but also soon ceased. A continuous suture from base to apex of right ventricle was made. The application of the suture was easier than in the smaller animals. Toilette of the pericardial sac, after which the latter was closed by continuous silk suture, and the muscles and skin sutured. Tracheotomy wound closed and iodoform collodion dressing applied to both wounds.

Two hours later the dog, though still under the influence of the morphine, was attempting to run around. The animal was found dead in his cage next morning, ten hours after operation. Post-mortem examination showed that from some cause, perhaps the restlessness of the animal, both pleuræ had been torn and both pleural cavities contained a small quantity of bloody serum.

LII. Large Dog. Heart exposed in the same manner as in Experiment LI. A wound 1.5 centimeters long, penetrating into the left ventricular cavity was made. An enormous hæmorrhage ensued. The wound was closed by pressure with artery forceps. The edges of the wound were then united by means of two deep, buried, and two superficially placed sutures. All the sutures were tied during systole. Pericardial sac and external wound closed as in LI. Death eight hours later. Post-mortem examination shows wound 1.5 ctm. in left ventricular wall to be firmly closed. Left

pleural cavity half-filled with fluid blood. When the superficial sutures were cut and the deeper parts of the heart-wound were examined, the buried sutures were found to have partially torn out through the heart-muscle.

LIII. Medium-sized Dog. Heart exposed by a procedure similar to that of Experiments LI and LII. Interrupted sutures were applied to numerous points on both ventricular walls in order to investigate the best method of applying and tying the sutures. Suture through whole thickness of left wall near base. This suture tears out at once, and necessitates the applications of two superficial sutures penetrating only the pericardium and superficial layers of myocardium. These hold. Three sutures passed through left ventricular wall and three through right ventricular wall. Two of the sutures on each side (a, b) tied during systole, the remaining one (c) during diastolic relaxation of the ventricles. Of these, two of the former (a, b) tear through the heart-muscle. Two sutures applied to replace these and tied in diastole; they hold. Pericardial layer incised for about two centimetres. The bleeding is considerable at first but ceases after compression with gauze. An attempt was made to observe the effects of heart tamponade. An incision 1 cm. long was made in the wall of the right ventricle and the opening in the pericardial sac rapidly closed by means of artery forceps. The bleeding was so great, however, that the sac burst in its lower and posterior part and the animal died in a few moments from the blood that escaped into the left pleural cavity.

Six experiments on rabbits, in which observations on the physiological irritability of the heart-muscle were made are not described in order to save repetition of what has been detailed in Part II, Series I, page 491.

A CASE OF ACUTE ENDOCARDITIS CAUSED BY MICROCOCCUS ZYMOGENES (NOV. SPEC.), WITH A DESCRIPTION OF THE MICROORGANISM.*

By WILLIAM G. MacCALLUM, M. D., and THOMAS W. HASTINGS, M. D.
(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

Clinical History. September 14, 1898, a German, aged 37 years, was admitted to the service of Dr. Osler, complaining of fever which had persisted since early in July. The history of the patient's family and his occupation were of no importance. In 1889 the patient was confined to bed three weeks with "rheumatic fever," though no joint symptoms were recalled. Otherwise the history was that of a healthy man of good habits.

July 4, a severe cold was contracted, fever appearing on the 6th and persisting until early in August when it subsided but reappeared about the middle of the same month, and for this relapse in supposed typhoid fever the patient was sent to the hospital. Severe frontal headache, stiffness and pain in the right shoulder joint, epistaxis, loss of strength, anæmia, and loss of 26 pounds in weight were noted during July and August.

Examination on admission showed a well-built, emaciated man, with a moderate grade of anæmia, and a temperature of 103° F. There was decrease in the area of cardiac dulness, and a pure diastolic murmur was detected over the body and base of the heart, suggesting disease of the aortic valves. The pulse also indicated incompetency of these valves. Over the abdomen were scattered a few erythematous spots thought at the time to be the rose spots of typhoid fever. The spleen was large, readily palpable and rather firm. The right shoulder joint which in July had been painful and stiff was now apparently normal. The urine showed a well-marked Ehrlich's diazo-reaction and a faint trace of albumin. During the first ten days in the hospital, anæmia, emaciation, fever, enlarged spleen and the cardiac condition of aortic incompetency

* A preliminary communication appeared in the Bulletin of the Johns Hopkins Hospital, 1899, x, p. 46.

were the most striking symptoms. At times stiffness and pain about the right shoulder joint were complained of, but there were at no time signs of inflammation about the joint.

The high temperature of 103-104° F. and a leucocytosis of 18,000 persisted. A suggestive Widal reaction also was present.

Though in a satisfactory condition on the evening of September 23, the next morning the patient appeared much more seriously ill. Delirium of the night previous had given way to stupor; anæmia was of extreme grade; the extremities were cold; and the appearance one of collapse. The lungs were quite clear but the heart, dilated to twice its previous size, had become rapid and irregular in action, and the physical signs of aortic disease were no longer recognizable. On the afternoon of September 23, the maximum cardiac impulse was readily located in the 5th left interspace, 9 cm. from the midsternal line, and the area of relative and absolute cardiac dulness was less than normal; on the morning of the 24th the maximum cardiac impulse was in the 6th left interspace 12.5 cm. from the midsternal line and the area of relative and absolute cardiac dulness had increased to twice that of the normal. During the night of September 24, the cardiac condition had somewhat improved and endocardial murmurs and a palpable thrill appeared at the apex suggesting both mitral and aortic disease.

At this time agar plate cultures from the blood were prepared on which within 48 hours grew colonies of what appeared to be a short-chained streptococcus, which subsequently on various media proved to be a micrococcus occurring mostly in pairs.

There subsequently occurred repeated attacks of collapse from cardiac failure up to the time of the patient's death on October 3, 1898.

October 1, three days before death, blood cultures were again prepared. On these cultures there grew the same micrococcus occurring mostly in pairs, the growths on various media corresponding to those obtained from the cultures prepared September 24.

Autopsy. Performed on the day of death, the body having been kept on ice and showing no evidences of post-mortem change. Description will here be given of only those organs which were the seat of pathological change.

Anatomical diagnosis. Subacute and acute ulcerative endocarditis of aortic and mitral valves; acute splenic tumor; septic infarctions in spleen and kidneys; embolic abscess in intestinal wall; bronchopneumonia; chronic diffuse nephritis of moderate degree.

Body emaciated, 184 cm. long; rigor mortis slight. Abdominal muscles of a deep red color; no excess of peritoneal fluid; peritoneal surfaces

smooth and glistening; a few old pleuritic adhesions: pericardial layers smooth.

Heart distended with liquid blood: weight 120 grammes. Nothing abnormal in the right heart. On the left side the mitral valve showed slight old thickening and in the middle of the edge of the aortic segment was a small, dark-red, fresh vegetation; many of the chordæ tendineæ had been ruptured, the ends of the ruptured cords being coated with fibrinous deposits; others were markedly thinned in the median portion, the adjoining parts being much thickened by fresh vegetations. The aortic valves were matted together by the exuberant vegetations which had formed upon them; a prolongation on the anterior coronary segment formed a flattened plate, which was apparently in part at least a mass of organized tissue, perforated by a hole about 2 mm. in diameter; the posterior coronary segment was surmounted by a rough mass of vegetations; the mitral segment was similarly covered with vegetations through which a ragged perforation was evident. From a point on the ventricular wall at the base of the posterior coronary segment to the base of the aortic segment of the mitral valve there ran a cord, like a moderator band, thickened in its median portion by beaded vegetations and entangled in the clots formed about the aortic vegetations. There were fresh vegetations also on the ventricular wall and on the wall of the aorta just above the valves.

Spleen, bound up in adhesions with the diaphragm and parietal abdominal wall, was much enlarged and very soft so that on attempting to remove it the capsule was ruptured in several places. Weight 470 grammes. The surface was peculiarly discolored over irregular areas which occupied a large portion of the capsule. Beneath some of these the substance was firmer than normal and on section elevated above the surrounding tissue, such areas corresponding to firm yellowish-white masses extending into the splenic pulp and sharply marked off by hæmorrhagic zones. All degrees of softening of such masses were present and the larger discolored portions of the capsule proved to form merely thin walls covering irregular cavities in the spleen filled with purplish-brown grumous fluid. These cavities also were delimited from the surrounding tissue by mottled zones of red and white. The relatively normal splenic pulp between the areas of infarction was much swollen and the Malpighian bodies and trabeculæ could be made out only with great difficulty.

Kidneys also presented superficially areas of purplish and grayish discoloration, of varying form and size, sharply demarcated from the surrounding cortex by lines of congestion and hæmorrhage. On section

these areas were seen to correspond with somewhat wedge-shaped masses varying in consistence and color. The smallest and evidently the freshest of these were firmer than normal, yellowish-white and elevated above the surface of the adjacent tissue; the larger and older masses were much softened and of a grayish-purple color throughout. In addition, the kidneys presented evidences of moderate chronic diffuse nephritis.

In the lower third of the *ileum* one of the lymphatic nodules in the mucosa appeared much swollen and reddened with a zone of hæmorrhage round about it. This nodule was quite firm and elevated several millimetres above the surface of the mucosa.

The left *lung* showed small bronchopneumonic patches throughout the lower lobe.

Microscopic examination. Sections of the diseased organs showed both acute and subacute morbid processes. Sections of the cardiac valves and vegetations showed a considerable curling and twisting of the valve proper. Thrombus masses surmounted the valves and formed the support for a fairly advanced growth of granulation tissue. The more superficial portions of the vegetations, however, consisted of platelets, fibrin and leucocytes, together with myriads of micrococci which, stained by Weigert's or Gram's method, formed dense bluish-black masses throughout the thrombus. Smear preparations from the vegetations showed the micrococci apparently unmixed with any other organisms and occurring often within leucocytes.

Sections through the discolored and softened areas in the kidney showed the typical picture of septic infarction with complete necrosis of the epithelium and the invasion of many polymorphonuclear leucocytes. The blood-vessels near the apex of the wedge were in many instances plugged with masses of micrococci. The firmer whitish masses both in the kidney and spleen were simple anæmic infarctions.

The small nodule in the ileum showed on section a mass of lymphatic tissue in the centre of which a small artery was completely occluded by a dense hyaline thrombus; about the vessel was an accumulation of polymorphonuclear leucocytes and nuclear fragments, together with a considerable exudation of fibrin; the neighboring vessels were also congested.

Cultures. Agar cultures in Petri dishes were made from the heart's blood, valvular vegetations, gall-bladder, splenic and renal infarctions, and other parts, and in all of these there appeared numerous, minute, pin-point, somewhat opaque, white colonies in the depths of the medium, and corresponding superficial colonies. The latter were also small, rarely exceeding the size of a pin's head. They were round in outline,

smooth, glistening, transparent, almost colorless or, on microscopical examination, perhaps slightly brownish, and slightly elevated above the surface of the medium. No other kind of colonies appeared on the Petri plates.

From the deep and superficial colonies obtained from the different organs a single species of microorganism was isolated and studied in all of its essential characters.

DESCRIPTION OF THE MICROORGANISM.

Morphology. Coverslips prepared from the original colonies and from the secondary tube-cultures showed a micrococcus in every way identical morphologically with that found in smears from the cardiac valves and with that isolated from the blood during life.

The organism is an extremely minute micrococcus, often somewhat elongated or elliptical in outline. It is much smaller than the Micrococcus lanceolatus. It occurs often singly, often in masses, but by far most frequently in pairs, two or more pairs being sometimes united into short chains, and indeed in cultures made in a hanging drop of bouillon chains of twenty or more members may occasionally occur, although in such a drop the usual grouping is in pairs. In the pairs or short chains the longer axis of the coccus is often transverse to that of the chain.

No motility is to be observed in the hanging drop other than the oscillatory motion common to all fine particles in suspension.

The organism stains well with the ordinary aniline dyes and by Gram's method remains deeply stained. No evidence of the presence of a capsule has been obtained either in cultures or in the tissues.

Cultural characteristics: Agar. Smears made in slanted agar tubes give a rather profuse, thin, slightly elevated growth along the line of the smear. The growth is somewhat moist and glistening, almost colorless; by reflected light, pale grayish-white; by transmitted light, transparent and of a somewhat smoky or brownish tint, old cultures showing this smoky discoloration more distinctly. The growth is not always, however, a diffuse flat expanse, but is often made up of a conglomeration of minute dew-drop-like colonies. The margins are somewhat crenated by the presence of larger, and sometimes

discrete, glistening colonies, slightly more elevated than those nearer the centre. Superficial, discrete colonies on agar may somewhat exceed one millimetre in diameter.

Glycerine agar. Cultures on glycerine agar grow more profusely but in other respects are identical with those on plain agar.

Ascitic-fluid agar. On ascitic-fluid agar the growth is distinctly more profuse and opaque than upon plain nutrient agar.

Glucose agar. The growth along the stab in glucose agar is moderately profuse but neither in stab cultures nor in cultures made in liquefied glucose agar, which is then allowed to solidify, is there any production of gas.

Bouillon. Broth becomes slightly clouded after twenty-four hours' growth, but in the course of three or four days the organisms settle to the bottom and form a whitish sediment leaving the overlying fluid clear. No indol is produced.

Sugar bouillon. In glucose and in lactose litmus bouillon (Smith's) in Smith's fermentation tubes acid is produced without gas, the medium becoming turbid in both arms and decolorized in the closed arm.

Potato. On potato, minute colonies appear and in about 36 hours become elevated, moist, of a somewhat dirty white color and pasty consistence, and after 72 hours confluent. Later the growth becomes rather dry and brownish. Occasionally there is no visible growth on potato.

Gelatine. In stab cultures in gelatine a somewhat opaque, granular growth extends along the line of stab and after about 36 hours a slight cupping appears at the surface and extends slowly downward, producing a tubular area of liquefaction which gradually involves the whole medium. Liquefaction is slower than in the case of Staphylococcus pyogenes aureus.

In gelatine plates the organism forms small pale granular colonies, yellowish by transmitted light, which float in small areas of liquefied gelatine.

Blood serum. On coagulated blood serum the growth is profuse and similar to that on agar, although perhaps somewhat less trans-

parent; along the line of smear the medium becomes somewhat translucent and is depressed, forming a definite groove below the surrounding surface. This change is similar to that observed in cultivations of Bacillus subtilis on blood serum. This partial liquefaction is accompanied, as a rule, with the production of a clear fluid and the breaking down of the solid medium.

Milk. The most characteristic cultural features of this organism appear in milk. In litmus milk of neutral reaction the immediate result of the growth is decolorization of the litmus within a period of four hours, a thin blue layer remaining at the surface. This decolorization is evidently due to deoxidation as the color returns to a certain extent on shaking the fluid with air, the returning color being, however, distinctly nearer to red than the original. Within twenty-four hours the milk becomes quite firmly coagulated, with the bluish or reddish layer still at the surface and the absence of color below. Next in time the upper layers of the coagulum become translucent and are gradually changed into a somewhat turbid fluid which is definitely red in the more superficial part and yellowish below. This softening and liquefaction of the coagulum progresses day by day, the layer of red fluid increasing in depth and the coagulum being transformed into a flocculent granular material floating in a yellowish liquid; finally, after several days, the whole coagulum is transformed and the red color extending into the depths stains the precipitate throughout; settling of this precipitate to the bottom of the tube at last leaves a clear straw-colored supernatant fluid overlying a dark red sediment.

These changes produced in litmus milk are absolutely constant and serve to distinguish this micrococcus sharply from related members of the pyogenic group of cocci.

Production of enzymes. Attempts were made to determine the nature of some of the metabolic products of the micrococcus.

Grown in sugar-free bouillon the reaction of the culture is alkaline, whereas in ordinary sugar-containing and lactose-containing bouillon it is acid. The acid reaction of milk cultures is, therefore, doubtless due to the production of acid by the decomposition of the milk sugar.

Experiments were made to determine whether in addition to the

formation of acid a milk-curdling ferment (Labferment, rennin, pexin) was produced in sufficient quantity to coagulate milk. Milk cultures, which had reached the stage of coagulation, were passed through a Pasteur filter to remove the bacteria and the resulting clear filtrate was added in small quantity to sterilized litmus milk. No appreciable change in the reaction of the milk followed this addition, but within 36 hours there was produced a coagulum quite as firm as that caused by the action of the living micrococci themselves. No decolorization nor acidification of the medium occurred. In the course of time the coagulum retracted somewhat, leaving a clear fluid overlying it. This experiment establishes the existence of a rennin-like ferment among the products of the micrococcus. The solution of the coagulated casein, as well as the liquefaction of gelatin and blood serum, indicate the formation of a proteolytic ferment related more or less closely to enzymes concerned in the digestion of proteids. The sterile filtrate, obtained by passing through a Pasteur filter milk cultures in which the liquefaction of the coagulum is in progress, was added to sterile milk and to nutrient gelatine for the purpose of observing the production of these changes without the presence of the organisms themselves. In these experiments the curdling of the milk proceeded as described above without much change in the reaction. On standing in the thermostat, this coagulum has of course a tendency to contract, but a further diminution in its size by the softening and liquefaction of its superficial layers is quite evident. The liquefaction is, however, not so complete as when the living organisms are present.

When a few drops of the filtrate are dropped upon the surface of sterile nutrient gelatine, this medium becomes slowly liquefied with the production of a clear fluid. Control inoculations in bouillon, milk and agar from these tubes of coagulated and peptonized milk and of liquefied gelatine, gave uniformly negative results, and coverslips from them showed no organisms.

The conclusion, therefore, is justified that this micrococcus produces both milk-curdling and proteolytic enzymes, separable from the bacterial cells and capable, when thus isolated, of producing their characteristic effects in milk and in gelatine.

Relation to oxygen. The micrococcus is a facultative anaërobe, the cultures grown in Buchner jars presenting quite as luxuriant a growth as those kept in the open air. Grown in hydrogen in a Novy jar, however, the colonies are rather more scanty and thinner.

Vitality. The organism is very hardy and tenacious of life. Agar cultures which stood in the laboratory from October until February and which were very much dried out furnished a profuse growth on a fresh agar tube. In this respect it obviously stands in marked contrast to Micrococcus lanceolatus which can be kept alive only with much difficulty.

Moderate variations in temperature seem to have no deleterious influence on the micrococcus. Indeed, it can withstand a temperature higher than that which is fatal to many micrococci. In order to fix the thermal death-point, portions of a bouillon suspension of the organism were sealed in Sternberg bulbs and heated for a period of five minutes each, to different temperatures. After exposure for five minutes to a temperature of 65° C., no organisms grew when the bouillon was transferred to the surface of an agar slant. After heating to 62°, only one or two colonies developed, but exposure to a temperature of 60° for five minutes produced hardly any retarding effect upon the growth of the organisms.

To antiseptics, such as carbolic acid and chloroform water, the micrococcus is considerably resistant, a relatively large precentage of antiseptic being required to kill all of the organisms in bouillon cultures.

Pathogenesis. The ordinary laboratory animals were inoculated in various ways with cultures and suspensions of the organism.

White mice. These animals, inoculated intraperitoneally with various quantities of thin suspensions of the organisms in bouillon, died in the majority of the experiments after periods varying from seven hours to three or four days, with evidences of general infection. Autopsy showed always a much swollen spleen and sometimes reddened lymph glands. The micrococci were recovered in pure culture from the heart, spleen, liver, and kidney. The dose given varied from 0.3 to 0.7 cc. of the suspension.

White mice were inoculated with a loopful of the solid culture also subcutaneously, but although they died after several days, the organism was not recovered from the organs. Inoculated subcutaneously with relatively large quantities of the bouillon suspension (0.8 to 1.8 cc.) they died within four to ten days, the organs showing the same changes as after intraperitoneal inoculation and yielding the cocci again in pure culture. No macroscopic lesion of the muscles near the seat of subcutaneous inoculation was visible.

Wild mice proved to be distinctly less susceptible than the white, although occasionally they succumbed to intraperitoneal inoculation with general septicæmia.

Guinea-pigs, white rats and *pigeons* showed very little susceptibility to inoculation.

Rabbits. A number of rabbits were inoculated, for the most part intravenously, but also intrapleurally and intraperitoneally. The latter modes of inoculation were followed by recovery of the animal. Indeed, rabbits appear, in general, rather resistant to this organism, since of eight rabbits inoculated only one developed a general septicæmia. Two of the rabbits showed at the site of inoculation at the time of death, abscesses which contained the micrococcus, and from one of them, as well as from the animal which died with a general infection, the organism was recovered from the contents of the distended urinary bladder.

One of the rabbits received 2.5 cc. of a thick bouillon suspension, containing particles of sterilized potato, into the ear vein. This rabbit became very ill and died after five and a half days, the micrococcus being recovered in pure culture from the blood and all the organs. The condition of the heart was most interesting. The mitral valve showed the presence of two quite firm vegetations, one on each leaflet, that on the aortic leaflet being the larger and measuring about 2 mm. in diameter. The thrombus mass was situated very near the edge of the valve and there was a propagated dark red clot running upward toward the base of the valve on the auricular surface. The smaller vegetation on the opposite leaflet of the mitral valve was situated somewhat nearer the base of the valve, and also on the

auricular surface. The papillary muscles appeared opaque and yellowish. The other valves, as well as the musculature, were apparently normal. Smears from the mitral vegetations showed the presence of the micrococci, unmixed with other organisms, and cultures from these vegetations gave a pure growth of the micrococcus.

Thus of seven rabbits inoculated three showed no ill effects, two died after several days with no apparent lesions at autopsy and no evidence of general infection (from these the organism was not recovered), one died of an intercurrent affection with a small abscess containing the micrococcus at the point of inoculation, one died with a large abscess at site of inoculation, the organism being recovered from the abscess and the distended urinary bladder, and finally one rabbit died with a general septicaemia, together with an *acute vegetative mitral endocarditis*, from which the organism was recovered in pure culture.

Dogs. Two dogs were also inoculated. One of these showed no ill effects, although 10 cc. of a thick suspension, containing particles of potato, were injected into the jugular vein; after sixteen days this animal was killed but at the autopsy the organs appeared quite normal and cultures from all parts were sterile.

In the case of the second dog, Rosenbach's procedure was adopted. The left carotid artery was opened under aseptic precautions, the aortic valves were torn through and lacerated by means of a long probe passed down the carotid, and the artery was then ligated on both sides of the opening. Two cubic centimetres of a thick suspension of the micrococcus in bouillon were then injected into the left jugular vein which was then ligated and the wound of the skin was closed with sutures. The dog appeared rather ill for a few days, but evidently improved slightly after the fourth day. Immediately after the operation there was detected a loud and distinctly diastolic murmur which persisted until the seventh day when the dog was killed with chloroform and an autopsy performed.

The spleen was found enlarged, congested and partly covered with a fibrino-purulent exudate. The other organs were macroscopically apparently normal with the exception of the heart. The right side of

the heart was normal. The aortic valves were the seat of an *acute and subacute endocarditis.* The probe had passed into the sinuses of Valsalva and had penetrated into the muscular tissue of the septum ventriculorum, running down 1.5 cm. just to, but not piercing, the endocardium over the septum. On section the muscle about this area was grayish and translucent in appearance with grayish opaque dots scattered over the translucent area. A second injury had occurred in the sinus behind the mitral segment of the aortic valve, the probe perforating the base of the valvular segment and passing into the ventricular cavity near the base of the mitral valve. About this opening there were on both surfaces of the valve granular translucent vegetations which extended upon the mitral valve and also upon the intima of the aorta just above the aortic valves. Coverslips from the vegetations and necrotic muscle showed numerous micrococci in pairs, many of them within leucocytes. Cultures from the same locations gave a pure growth of the micrococcus, as did cultures from the blood, liver, spleen, and kidney.

Toxine. Attempts were made to obtain a toxine from cultures of this organism, but so far with little success. A priori, it would not be expected that an organism so closely resembling in its general properties the pyogenic group of cocci would produce a toxine of high virulence. The wide dissemination of the cocci observed in the cases of general infection would also point to the same inference, and this suggestion is supported by the few experiments thus far made relating to this point.

A culture, made in a large quantity of sugar-free bouillon and allowed to grow in a Roux flask for three weeks, was killed with chloroform. Ten cubic centimetres of this killed culture, after evaporation of the chloroform, were injected into the ear vein of a rabbit, a control rabbit receiving a similar injection of the same quantity of sterile sugar-free bouillon. Neither rabbit showed elevation of temperature or any other ill effect following the injection.

SUMMARY.

From a case of acute endocarditis of the aortic and mitral valves with infarctions in the spleen and kidneys a micrococcus was twice

isolated in pure culture from the blood during life and was demonstrated after death both microscopically and in pure culture in large numbers in the valvular vegetations, the infarctions and other parts. No other species of microorganism was found.

This micrococcus is very small, occurs mainly in pairs, sometimes in short chains, stains by Gram's method, grows in small, pale, grayish-white colonies on gelatine and agar, at first clouds bouillon, which then becomes clear with a whitish sediment, does not produce gas in glucose media, liquefies gelatine slowly and to some extent also blood serum, and is especially characterized by its behavior in milk, which it acidifies, coagulates and subsequently liquefies. It produces a milk-curdling ferment and also a proteolytic ferment, each of which is separable from the bacterial cells. It remains viable for months in old cultures and is tolerably resistant to the action of heat and antiseptics.

The micrococcus is pathogenic for mice and rabbits, causing either abscesses or general infections. Typical acute vegetative endocarditis was experimentally produced by intravenous inoculation of the organism in a rabbit and a dog, and the cocci were demonstrated in pure culture in the vegetations and other parts of these animals after death.

Although the micrococcus here described has some points of resemblance to the pneumococcus and Streptococcus pyogenes on the one hand and to the pyogenic staphylococci on the other, it is readily distinguished from each of these species by cultural features which have been described and which are so obvious that the differentiation of these species from our micrococcus need not be discussed in detail. We have searched through the records concerning microorganisms described in association with endocarditis and other diseases, as well as those isolated from water, soil and other sources, and have been unable to find a description of a micrococcus identical in all particulars with that here described. Such points as staining by Gram, liquefaction of gelatine, coagulation and peptonization of milk, served singly or in combination to distinguish our micrococcus from other forms which

in some respects might resemble it.* We feel justified, therefore, in recognizing this organism as a new species and from its fermentative properties propose for it the name "Micrococcus zymogenes."

Micrococcus zymogenes must be added to the already considerable list of bacteria which have been found as the specific infective agents in endocarditis. That it was the cause of this affection in our case was conclusively demonstrated by its repeated isolation in pure culture from the blood during life, by its presence in pure culture and large numbers after death in the cardiac vegetations, the infarctions, and other parts of the body, and by the experimental proof of its pathogenic properties, and notably its capacity to produce vegetative endocarditis by intravenous inoculation in animals.

* Dr. Norman MacL. Harris in this laboratory isolated in May, 1898, from material removed from an old cesspool in Baltimore, which had not been evacuated for twenty-two years, a micrococcus which in morphological and cultural characters is so similar to Micrococcus zymogenes that we at present believe the two organisms to be identical. The cesspool coccus at present liquefies gelatine and peptonizes milk somewhat more slowly than our coccus, but otherwise its cultural characters are indistinguishable from those of the latter, and morphologically the organisms are identical. Its pathogenic properties have not yet been tested.

A STUDY OF THE NEURONE THEORY.*

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PLATES XXIII AND XXIV.

The opinion is now widely accepted, that the nerve cells throughout the central nervous system are independent structures. The cell processes, it is thought, invariably run out to end free, and one nerve cell has no further connection with any other than that of mere contact. This, in brief, constitutes the neurone theory which is so vigorously maintained by von Lenhossék, Golgi, Ramón y Cajal and others.

The claim for the independence of the nerve cells is based almost entirely upon the use of Golgi's silver impregnation method, and the embryological researches of His. The investigations of the latter may indeed show that all the dendrites of a nerve cell develop from the neurocyte, and that they do not anastomose with each other; but when in mature cells such anastomoses are demonstrated anatomically, the observations of His lose some of their significance. But notwithstanding the fact that Golgi's method demonstrates only individual elements, and these for the most part only imperfectly; and notwithstanding the fact that the pictures presented are so crowded with artefacts as to cause every statement concerning the histology of the cells to be made with the greatest reserve, still as a result of its use, the old observation of Gerlach (1), that the nerve cells anastomose with each other, has been discarded, and the more modern neurone theory substituted.

Held (2) does not believe in the neurone conception and maintains that anastomoses between nerve cells are demonstrable by Golgi's method. My own observations have led to a similar conclusion.

* A preliminary communication was read before the Chicago Pathological Society, April 10, 1899.

Even after obeying von Lenhossék's instructions to choose for investigation only single, isolated impregnated cells, anastomoses have been demonstrated, whose genuineness, to my mind, is not to be doubted. My observations are based upon a study of the cerebral cortex, basal ganglia, and motor spinal ganglion cells of the white rat and of the spinal cord of man.

In the cerebral cortex, the pyramidal cells are usually connected in a sort of end-to-end manner, the large dendrite of one cell passing upward and into the body of another cell directly above (Plate XXIII, Fig. 1) or above and to one side of it. This same condition has been described by Růžička, who, however, working with toluidin blue, employed a totally different technique. Thus two entirely different methods have given the same results. A few times I have found these pyramidal cells to be connected by transversely running dendrites, which join together cells lying in about the same plane in the cerebral cortex (Plate XXIII, Figs. 2 and 4). In the basal ganglia, the cells are connected in various ways, the usual arrangement consisting of short protoplasmic bridges which connect the two cell bodies (Plate XXIII, Fig. 5). The spinal ganglion cells furnish interesting pictures of anastomosing cells. For the most part two cells are found connected by a common dendrite; but three are not uncommon, and in one case an anastomosis between five cells was noted (Plate XXIII, Fig. 3).

Since, however, the upholders of the neurone theory will in no case admit an anastomosis which is demonstrated by Golgi's method (condemning such in every case as only apparent), we are driven to adopt another method to settle this dispute.

Dogiel, who has demonstrated anastomoses between the nerve cells of the retina, has employed Ehrlich's method of the intravenous injection of methylene-blue. Bethe (3) has used molybdic-acid toluidin blue, while Růžička (4) has worked with plain toluidin blue; both have found that the nerve cells not only come into contact with each other, but actually anastomose.

In my investigations of this subject, I have employed, besides Golgi's method, the following modification of Nissl's method:

(*a*) The tissue is hardened in a 10 to 20 per cent solution of formalin, cut with a freezing microtome, or dehydrated, embedded in celloidin or paraffin, and sectioned.

(*b*) The sections, which should not be too thin, are stained from one to twenty-four hours in a solution of Grübler's soap methylene-blue which has been diluted with an equal amount of water.

(*c*) The specimen is rinsed in water and dehydrated and differentiated in aniline-oil alcohol (1-20).

(*d*) Cleared in oil of cajeput and mounted in Canada balsam.

I consider this method superior to Ehrlich's intravenous injection of the dye for this purpose, as the pictures obtained are more intensely stained. Růžička's method is, I think, improved by the use of formalin instead of corrosive sublimate, as formalin acts as a powerful mordant for the dye, and this permits of a longer period of differentiation in alcohol, making the resulting pictures stand out more prominently.

The nerve cells in specimens colored by the method I have given are overstained. The cell processes are stained for long distances from the cell body, as are also the branchings and subbranchings. Furthermore, all neighboring structures are stained, so that the true relation of these to the neurones can now be studied to better advantage. The Nissl bodies are plainly visible in the cells and dendrites, so that the identity of these structures is never doubtful. The nucleus appears usually as a somewhat lighter blue spot in the centre of the cell body.

It is now an easy matter to see that the nerve cells are not separate individuals, but frequently anastomose with each other. The relation of two cells is not always one of mere contact only, but one of actual connection between the protoplasm of one cell and that of the other.

The two cell bodies are in these cases connected with each other by a common dendrite (Plate XXIV, Figs. 6, 7, 8), which is in every way similar to that ordinarily observed. The process arises from the one cell by its usual, broad, wedge-shaped base, and its connection with the other cell is marked by a similar base. The Nissl bodies can be traced throughout the entire length of the dendron. The connecting dendrite is usually a short, protoplasmic bridge (Plate

XXIV, Fig. 6), but quite often extends to a greater length (Plate XXIV, Figs. 7 and 8). There is never any doubt as to whether the anastomosis is a true or only an apparent one. The dendrites are not opaque as in Golgi's method, but translucent, so that in every case it can be easily seen whether the protoplasmic processes really connect with each other or only touch each other. Thus far I have found anastomoses only between cells of the same group, and never between cells of different groups, an observation which is in harmony with that of Růžička.

Unfortunately the methylene-blue staining does not lend itself well to photomicrography, so that I have been forced to use drawings to illustrate my observations.

Sala (5), working with Golgi's method, has put forth the claim that the dendrites embed themselves in the blood-vessels. His position has been substantiated by Růžička, but is severely criticised by von Lenhossék. That some of the dendrites do actually embed themselves in the walls of the capillaries, is, to me, an established fact. The tip of the cell process, before it connects with the wall of the capillary, widens out into a wedge-shaped end (Plate XXIV, Fig. 9). The occurrence of this condition leads me to believe that the dendrites have, besides the generally accepted nervous, also a nutritive function.

As the result of this study, I would point out the following:

First. The neurone theory, in so far as it claims the absolute independence of the neurones is an untenable one, as anastomoses between them have been found.

Second. The dendrites, which are generally believed to have but nervous function, may have also nutritive function, if such an inference is permissible from the existing anatomical relations, which show some of the dendrites embedding themselves in the walls of the capillaries.

DESCRIPTION OF PLATES XXIII AND XXIV.

PLATE XXIII.

Fig. 1. Photograph showing anastomoses between the pyramidal cells of the cerebral cortex of white rat. Method of Golgi.

Fig. 2. Anastomosis between the nerve cells of the cerebral cortex of white rat. The dendrite seen running upward and to the right from the

upper neurocyte is the continuation of the delicate cell process which is seen to the right of the heavy common dendrite. Golgi method.

Fig. 3. Anastomoses between five motor ganglion cells of the dorsal spinal cord of the white rat. Golgi method.

Fig. 4. The bodies of two pyramidal cells of the cortex connected by a common dendrite. Brain of white rat. Golgi method.

Fig. 5. Two nerve cells of basal ganglia connected by common dendrite. Golgi's method.

PLATE XXIV.

Fig. 6. Two motor spinal ganglion cells connected by short protoplasmic process. Methylene-blue staining.

Figs. 7 and 8. Motor spinal ganglion cells from cervical region of human spinal cord. Methylene-blue staining.

Fig. 9. A dendrite embedding itself in the wall of a capillary. Human spinal cord, anterior horn cell of cervical region. Methylene-blue staining.

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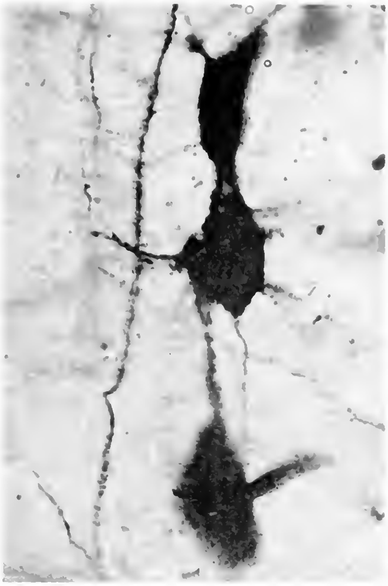

FIG. 1

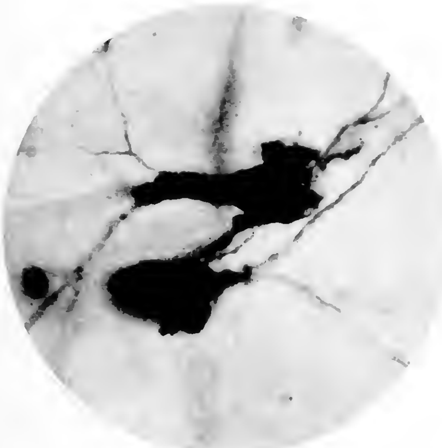

FIG. 2.

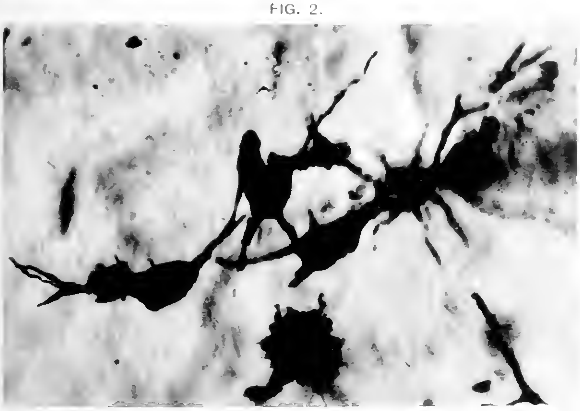

FIG. 3.

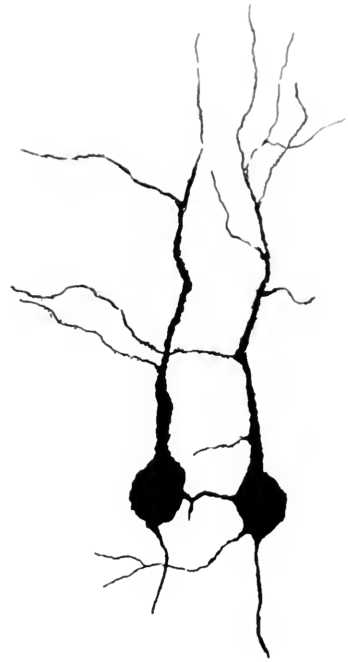

FIG 4

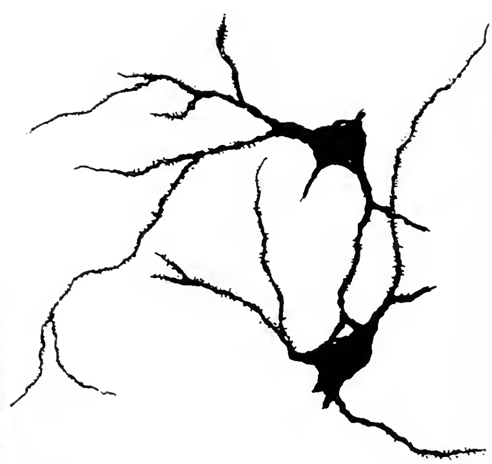

FIG 5.

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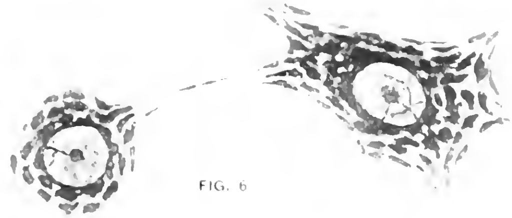

FIG. 6

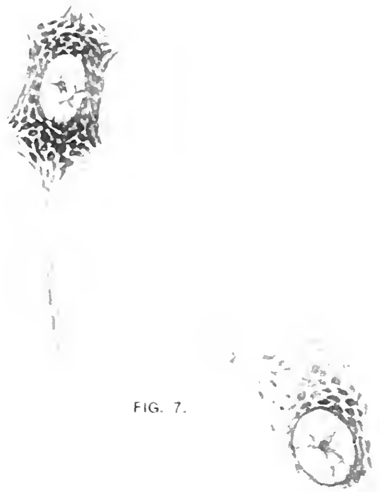

FIG. 7.

FIG 8

FIG 9.

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A CONTRIBUTION TO THE SUBJECTS OF CHRONIC INTERSTITIAL NEPHRITIS AND ARTERITIS IN THE YOUNG, AND FAMILY NEPHRITIS; WITH A NOTE ON CALCIFICATION IN THE LIVER.

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PLATE XXV.

Chronic nephritis in children and adolescents is a condition which has been much overlooked. Especially is this true of the chronic interstitial type (contracted kidney). Dickinson was of the opinion that the latter condition was not infrequent in children. Heubner (1), who has lately written a monograph upon this subject, from which many of these introductory remarks are cited, states that of 251 cases of chronic nephritis which he observed in Leipsic, 214 occurred in adults and 37 in children. Later, in Berlin, he saw 28 cases in children, mostly after scarlet fever. Of these 65 cases, there were 3 of parenchymatous nephritis, 4 of contracted kidney and 5 of chronic hæmorrhagic nephritis (Wagner). Of 33 cases of chronic interstitial nephritis observed by Bartels (2), one occurred at the age of 18, one at 19, and two at 20. Of Dickinson's (3) 308 cases, one occurred between 11 and 20 years, and 24 between 21 and 30 years.

From these figures it is seen that under the age of 20, chronic interstitial nephritis is uncommon, but not so rare as the omission of reference to it in many text-books on children's diseases would indicate. The condition is even more frequent than these figures would lead us to infer, as many of the cases are overlooked and treated for anæmia for a long time. This is especially true of the subacute cases of productive nephritis described by Delafield (4).

Of the interstitial cases, which alone interest us here, Heubner states that there are but thirty on record in which post-mortem examinations were made. Besides these, there are numerous other instances reported in which the patients were not observed until the fatal termination, or where no autopsy was performed. To these cases, Heubner adds four from his

own experience; three in males, aged respectively 14, 9 and 24, and one in a female aged 11. Of these four cases, however, one was probably of amyloid disease. In none of these cases is there any post-mortem examination recorded. In all the cases which Heubner cites the disease was very insidious, but was easily discovered upon properly examining the patients. As a rule the disease lasted for many years. It can, however, be rapid in its course, as in Crooke's (5) case, where, at the autopsy on a boy nine years old, who died on the 68th day after the onset of scarlet fever with diphtheria, the kidneys were found to be distinctly granular. Delafield (4) has drawn especial attention to these rapid cases (acute productive nephritis).

According to Heubner, there are seven cases in the literature in which there is no history of a previous acute attack, so that they can be looked upon as instances of primarily contracted kidneys. These are the cases of Barlow (6), Bull (7), Filatoff (8), Morell-Lavalée (9), Oppenheim (10), and Förster (two cases) (11). The case reported by Morell-Lavalée is of a special type, as the condition was complicated by a cirrhotic liver. In the other cases, which died at the ages of 5, 6, 10, 13, and 14 years respectively, it is noted that the children were always weak, of capricious appetite, pale, and had weak resisting powers.

Accidentally, or at the occurrence of a slight œdema, the albumin in the urine was discovered. It is, of course, possible, as Heubner states, that these children had had an attack of acute nephritis in earliest infancy or childhood, so that the question of the occurrence of a primarily contracted kidney in children must remain an open one. Of the four cases which Heubner has seen, one is probably a case of amyloid kidney as stated above, and two are probably secondary cases. Only one is possibly a primary case. This is that of a boy aged 9 years, who presented typical symptoms of chronic interstitial nephritis, but of whose history only a part is known.

It may be of interest to give details of some of the reported cases:

Barlow's case was that of a girl aged 6 years. She suffered from headache, pallor and vomiting, and died with uræmic symptoms. At the autopsy there was found a very marked chronic interstitial nephritis. The right kidney weighed 13 grammes and measured 5x4x0.5 cm. The left weighed 21 grammes and measured $6 \times 3\frac{3}{4} \times 1\frac{1}{4}$ cm. The arteries were thickened.

Bull's case was that of a girl thirteen years old. The left kidney weighed 20 grammes, was 5 cm. long and 3 cm. wide. The right measured $7\frac{1}{2} \times 4\frac{1}{2}$ cm.

Filatoff's case was of a girl aged 12, who was always weak and passed

very much urine. In her 11th year, she had an apoplectic attack with left-sided hemiplegia. Later she had headache and vomiting and began to lose her eyesight. An albuminuric retinitis was found. Before her death she became slightly œdematous and had a severe nasal hæmorrhage. The urine was clear, 1500 cc. daily, and slightly albuminous. The arteries were tense and hard, the apex beat was exaggerated and situated below and to the left of the normal position. The autopsy showed granular kidneys, hypertrophy of the left ventricle, endarteritis of the aorta and the vessels at the base of the brain, and an old hæmorrhagic extravasation in the right corpus striatum.

In Lavalée's case, a child 5½ years old suffered from ascites with fever At the post-mortem examination there was found cirrhosis of the liver, spleen and kidneys.

We have not been able to consult Oppenheim's article.

Förster's two cases occurred in the same family. The boy died at the age of 9½ years, after an illness of 5 years' duration. The autopsy showed typical contracted kidneys. The girl died at 8¼ years, having been ill 3½ years. Several weeks before her death she developed a hæmorrhagic diathesis, having several severe hæmorrhages from the mucous membrane of the mouth. An autopsy was not performed. Both cases during life resembled cases of diabetes insipidus.

To these seven cases gathered by Heubner, making eight with his own case, we may add a few others which we have found. Westphal (12) reports the case of a man aged 24 years in which there was found an atrophic kidney on one side and a contracted kidney on the other. There was no history of any previous illness and the patient succumbed after having been sick a very short time. It is of interest to note that Curschmann had diagnosed the existence of a contracted kidney with the probability of one kidney not functioning. Baginsky (13), in his text-book, mentions a case in a girl aged 4, which stimulated diabetes insipidus. There is no autopsy reported. Ashby and Wright (16), mention two cases which seem to belong in this group. They both occurred in girls, one 11½ and the other 10½ years. v. Buhl reported a case in a child of one and one-half years (cited by Eichhorst (28)).

It is a remarkable fact that even congenital cases have been described. Baginsky (13) mentions two such occurrences in his experience but gives no details. Weigert (14) describes briefly the following case: At the autopsy performed on a child six weeks old, who had suffered from marked cyanosis, there was found an atresia of the pulmonary artery, an open septum and hypertrophy of the right ventricle. The kidneys measured 1.7x1x0.5 cm. and 2x1.1x0.4 cm. The cortex was narrow and

the substance pale and firm. There were small cysts on the surface. Microscopically, there were found fairly normal parts with fatty degeneration of the epithelium and areas of marked chronic interstitial inflammation. The epithelium of the Malpighian tufts still consisted of large cells, and, like the cortical cells, showed fatty degeneration. Hellendall (14*a*) has reported two instances of contracted kidney, probably of congenital origin, in infants, the one six months and the other two years old.

The following case, which was observed at the Mount Sinai Hospital, adds another to this list of cases, and it is so interesting from many other points of view that it is considered worthy of publication.

Ida W., school-girl, aged 14 years. Admitted January 13, 1898. *Family history* is of special interest and will be given in detail subsequently (p. 554).

Previous history: The girl has always been weak and under-sized. She generally stayed at home because running around on the street made her short of breath. She has never menstruated. One year ago, her face and feet were swollen during the whole winter. She always felt better during the summer. For some time past she has had a cough with expectoration. When six months old she suffered from an attack of gastro-enteritis of a few days duration. She has had no other known acute illness.

Present history: One week ago, she became very much frightened at a fire in the neighborhood at 2 P. M. She was apparently well until 11 P. M., at which time she went to sleep. The next morning it was noticed that the left side of the body was paralyzed. She complained then and still does of headache. She had fever for the first time three days ago, and two days ago she coughed up a small quantity of blood. Urination and defecation are stated to be normal. Her intelligence and memory have not been impaired. The paralysis has improved somewhat; she has twitchings of the entire left side of the body.

Physical examination: Under-sized girl, poorly nourished, with a dirty, anæmic color. The tongue is moist and coated. There are no defects in the teeth and there is no lead-line present. There are no signs of any corneal inflammation, and no changes in the ears. She has a left-sided hemiplegia with some rigidity. Sensation is unimpaired in the upper but diminished in the lower extremities. *Lungs,* negative. *Heart,* dulness extends from 1 cm. to the right of the sternum to 5 cm. to the left of the mammillary line. The upper border is at the third

rib. The apex beat is in the fifth space in the axillary line. It is forcible and fairly localized. The heart's action is tumultuous and forcible. There is a systolic murmur over the mitral area, transmitted a short distance to the left. Over the aortic orifice there is heard a systolic murmur transmitted up and down the sternum and to the vessels of the neck. The second aortic sound is accentuated and reduplicated. The radial *pulse* is tense, the artery is very much thickened and slightly tortuous. The *liver* dulness extends from the fourth space to the free border of the ribs. *Spleen*, negative. *Abdomen*, negative. The right pupil is slightly larger than the left.

Subsequent history: January 12, 1898. Pulse 120. Respiration 32, temperature 101.8° F.

January 13. Urine neutral, 1010, clear, and contains albumin—1.4 grammes to the litre (Esbach); urea $6\frac{1}{2}$ grains to the ounce; hyaline and granular casts and a few pus cells are present. The quantity of urine could not be measured because the urination was involuntary. She has some cough and complains of pain in her stomach.

January 14. Temperature normal; labial herpes; patient somewhat soporose.

January 15. Urine contains albumin, red and white corpuscles, but no casts.

January 17. The movements from the bowels contain some blood.

January 19. Urine again shows hyaline and granular casts.

January 20. Mental condition has improved. Paralysis unchanged. Pain in abdomen less marked.

January 30. Marked restlessness.

February 3. Temperature elevated to 103°. She complains of pain in the left hypochondriac region. By percussion the spleen is found to be enlarged. It can be felt and is tender.

February 5. Temperature 104.6°. Has severe coughing spells. Over the first four interspaces on the right side anteriorly and posteriorly are numerous crepitant râles, increased voice and breathing and a dull percussion note.

February 6. Temperature 102°.

February 7. Over right lower lobe behind there is bronchial voice and breathing. Severe abdominal pain. Temperature 100°.

February 8 to 10. Patient's condition worse. She lies in a semicomatose condition and complains of severe abdominal pain. The hemiplegia has remained unchanged. The physical signs on the right side have partially cleared up. Electrical examination shows an almost com-

plete loss of reaction to the faradic current in both the left upper and lower extremities.

February 15. Ulcers found on the upper and lower gums with necrotic, easily bleeding bases.

February 18. Over the upper lobe of the left lung is an area of dulness, increased voice and breathing, crepitant râles. Hæmoptysis.

February 23. Necrotic areas in the mouth have increased in size and number. Ophthalmoscopic examination shows retinal hæmorrhages.

February 25. Continued hæmoptysis with signs of consolidation of a large area in the left lower lobe posteriorly.

February 26. Temperature suddenly drops to 96.4°. The patient had very severe pain in the abdomen and passed a large amount of blood with the stool. Pulmonary œdema.

February 27. Temperature still remains subnormal. Marked tympanites. Subcrepitant and crepitant râles heard in front and behind below the second rib down to the base on the right side. Râles heard yesterday have almost entirely disappeared. Urine contains albumin, 16 grammes to the litre. Urea 4 grains to the ounce. Temperature subnormal.

March 3. Very marked pericardial friction sounds heard even at a distance from the patient.

March 5. Crepitant râles over the upper part of the left chest with bronchial voice and breathing. Temperature 94° to 96°.

March 6. Return of the pulmonary œdema. Exitus.

Clinical diagnosis: Chronic interstitial nephritis. General arteritis. Atheroma of aorta and aortic valve. Cardiac hypertrophy. Cerebral hæmorrhage. Hæmorrhages into the lung and spleen. Hæmorrhages in the area of distribution of the superior mesenteric artery. Fibrinous pericarditis.

Post-mortem examination: Five hours after death. Body very much emaciated. Slight general œdema, more marked on the left side of the body, with marked œdema of the feet.

Lungs: Slightly adherent at the apices. Moderate pulmonary œdema. Both lungs are very firm. In the right upper lobe there is a very dense non-crepitating area which is infiltrated with blood. In the right lower lobe there is a similar smaller triangular area. In the left upper lobe, are several patches of consolidation and one large hæmorrhagic area. In the left lower lobe, there is a large recent hæmorrhage. There is marked subpleural emphysema of both lungs.

Heart: On the anterior aspect there is very much fresh fibrin; the left ventricle shows marked hypertrophy, the thickest part of the wall

measuring $3\frac{1}{2}$ cm. and the thinnest 2 cm. The aortic valves show moderate atheroma. The flaps of the mitral valve are slightly thickened. The wall of the right ventricle is somewhat hypertrophied. The aorta shows patches of atheroma especially around the openings of the coronary arteries. The coronary arteries are markedly atheromatous. On the endocardium between the aortic and mitral valves, and on one cusp of the mitral, are patches of marked atheroma. The cardiac muscle is pale but firm.

Spleen weighs 80 grammes; measures $5 \times 8\frac{1}{2}$ cm. Malpighian bodies are very distinct. Almost the entire spleen is taken up by a large hæmorrhage.

Kidneys, small, red. The right weighs 59 grammes and measures 9x4x3 cm. The left weighs $34\frac{1}{2}$ grammes and measures $6\frac{1}{4} \times 3\frac{1}{2} \times 2\frac{1}{2}$ cm. The capsules are very adherent; the surface irregularly granular. The kidney substance is firm; the cortex is very narrow. The pelves of both kidneys are markedly enlarged, and surrounded by fat. The markings are very indistinct. On the surface of the kidney are enlarged veins, and some of the glomeruli can be indistinctly seen as white dots. The left kidney is more markedly affected than the right, the parenchyma being only 0.5 cm. wide.

Stomach: Catarrhal inflammation. A few hæmorrhagic erosions near pylorus.

Intestines: There is a large hæmorrhage in the mesentery. No emboli nor thrombi can be found in the mesenteric arteries or veins or their branches. The superior mesenteric artery is markedly atheromatous. The small intestine shows catarrhal inflammation; the contents are mucoid and bloody. The large intestine shows catarrhal inflammation with marked injection of the walls.

Pancreas is indurated.

Liver somewhat enlarged, is fairly firm, shows chronic congestion and some increase in the connective tissue. In the right lobe on the anterior and inferior surfaces are triangular areas reaching to the surface in which there is a firm deposit in and along the vessels looking as if the vessels were firmly thrombosed.

The brain could not be examined.

Microscopical examination: Lungs show the changes characteristic of brown induration with a fair amount of increase in the interstitial connective tissue. The lower lobe shows areas where the air-vesicles are filled with epithelial and pus-cells and numerous red blood-corpuscles. The right upper lobe shows the same condition with a larger and more recent hæmorrhage. The left upper lobe contains a large area of

croupous pneumonia. The left lower lobe shows marked recent infiltration with red blood-corpuscles.

Liver: The capsule is thickened. The capillaries are dilated and there is a little increase in the interstitial connective tissue. The liver cells show marked degeneration and pigmentation. There are small hæmorrhages under the capsule. The walls of the hepatic veins are thickened and the veins themselves are dilated. The arteries show marked obliterating endarteritis. Scattered throughout the parts of the liver which showed such a peculiar condition macroscopically, are irregular areas (Plate XXV, Fig. 1) which stain very darkly with Delafield's hæmatoxylin and look necrotic. These areas seem to follow the branches of the hepatic artery, surrounding and sometimes obliterating the lobules. On the addition of dilute hydrochloric acid to the unstained specimen, these areas become much paler without the evolution of gas bubbles, and when thoroughly washed out and then stained with the same solution, they appear more homogeneous and stain with about the same intensity as the surrounding parts of the liver. They are, therefore, areas which have become impregnated with lime salts. The absence of any evolution of gas bubbles on the addition of the acid shows that they consist of calcium phosphate.

Spleen shows the changes of acute inflammation. The capsule is thickened and there is an increase in the interstitial connective tissue. The pulp is infiltrated with red blood-corpuscles. The arteries are the seat of a marked obliterating endarteritis.

Kidneys (Plate XXV, Figs. 2 and 4): The right kidney shows a marked chronic interstitial nephritis. In some areas the renal parenchyma is entirely replaced by fibrous tissue. Many of the glomeruli are converted into fibrous balls. Some of the tubules are dilated, others are atrophied; some contain casts, others granular material or blood. The epithelium shows marked degeneration. There are small scattered hæmorrhages present and signs of chronic congestion. The arteries show marked obliterating endarteritis (Plate XXV, Figs. 2 and 3), many of them being entirely closed. Scattered throughout the kidney are very small areas similar to those which have been described as occurring in the liver. The left kidney shows the same changes as the right, and in even more marked degree.

Pancreas contains areas of infiltration with round cells between the lobules, and in some places in the centre of the lobules.

Coronary arteries: There is extreme thickening of the intima, fibrous metamorphosis of the media and extensive calcific deposits and necroses in the intima (Plate XXV, Fig. 5). One of the smaller peripheral

arteries shows, microscopically, a thickening of all the walls; the intima contains calcified and necrotic areas, and there are broken-down thrombi attached to it. The media is almost entirely replaced by connective tissue. There are small hæmorrhages in the adventitia. *The superior mesenteric artery* likewise shows irregular thickening of the intima and of the media with small calcific areas in the intima.

Anatomical diagnosis: Pulmonary œdema; brown induration of lungs; hæmorrhages into lungs, spleen and mesentery; lobular pneumonia; cardiac hypertrophy; general chronic arteritis; chronic congestion of the liver, with calcific deposits; acute splenic tumor; catarrhal inflammation of the gastro-intestinal tract; chronic interstitial nephritis (small red kidney).

Remarks on the results of the post-mortem examination.—The kidneys from this case are among the smallest that have been recorded in the chronic nephritis of early life. In a few of the other recorded cases, the size of the kidneys has been as follows: Handford (15), 12-year-old girl, right kidney, 60 grammes; $8\frac{1}{2}$x$4\frac{1}{2}$x$2\frac{3}{4}$ cm. Left kidney, 15 grammes; 5x4x2 cm. In Ashby and Wright's (16) cases, from the one case of a girl $11\frac{1}{2}$ years old the right kidney weighed 75 grammes, the left kidney, 22 grammes. The kidneys from the other case weighed together 45 grammes and each measured 5 cm. in length. In one of Kidd's cases (17) one kidney weighed 60 grammes and the other 30 grammes.

The change in the liver, which is reproduced in Plate XXV, Fig. 1, is certainly very striking. In places it made the impression that the process began in the walls of the arteries and that then the parts supplied became degenerated and infiltrated with lime salt. The occurrence of such calcification in the arteries, beginning in the intima, was demonstrated in the kidneys and is illustrated in Plate XXV, Figs. 2 and 3.

The presence of calcified areas in the liver is extremely rare. Babes mentions an instance in an article on the technique of staining with safranin.* The liver described by him was obtained from a patient who suffered from tuberculosis of the femur and the hip-joint.

* Virchow's *Archiv*, 1886, cv, p. 511.

None of the other organs showed similar changes. The liver was large, grayish-brown in color, and presented grayish-yellow patches corresponding to the central veins, so that it looked like a nutmeg liver of a peculiar character. It felt sandy, and cutting it caused a grating sound. The deposit proved to be composed of lime salts in and around the central veins and their branches and in the surrounding cells. Around these areas the cells had lost their nuclei and their contours were obliterated. Babes considered the condition to be due to lime resorption from the bone disease and its later deposition in the liver. Virchow thought likewise, but the exact manner of its occurrence could not be determined.

Similar, but smaller and fewer areas of calcareous impregnation, were found in the kidneys and in the walls of many of the vessels. Figure 5 (Plate XXV) shows the deposits in the walls of the coronary artery. It also shows the other changes noted in the report of the microscopical examination. In Fig. 4 (Plate XXV) are shown the changes in the kidney, the part represented, however, not being that most markedly affected. The large number of vessels almost or entirely closed is very striking. As stated above, an examination of the brain was not permitted, but it is probable that the hemiplegia which marked the onset of the symptoms was due to a right-sided hæmorrhage.

For the sake of completeness we may note here that a blood culture was made 14 hours before death. A pure culture of Staphylococcus albus was obtained, and after death the same organism was cultivated from the heart's blood. We lay no stress upon this observation, as the agony was very prolonged and the bacteræmia may have been due to an agonal invasion.

General Remarks.—(1) As to the diagnosis of the main conditions, only a few words are necessary. At first sight the case might have been considered as primarily cardiac in nature with secondary renal changes of the chronic interstitial type. The marked hypertrophy of the heart, however, with the distinctly accentuated second sound caused us to suspect a primary affection of the kidneys. The finding of the diseased radial vessels supported this view. It must be remem-

bered, however, that a similar clinical picture can be produced by disease of the vessels without involvement of the kidneys. This fact, which has not received proper recognition, is strikingly illustrated by a case recorded by Hawkins.* As this case resembles ours to a great extent, we present it in detail:

A girl, aged 11 years, in the summer of 1891, had a week's illness, probably attributable to infarctions of the lung. In January, 1892, similar symptoms again appeared, the pain being first on the left side and later on the right. The cough, with blood-stained sputum, persisted up to the end of the illness. February 7, the legs became œdematous and later the face. The urine showed a trace of albumin, no casts, and was diminished in quantity. February 25, the urine was bloody and contained a little albumin, and the patient vomited. The child died after three weeks from what seemed to be uræmic poisoning, the case being considered one of acute nephritis. There was no syphilitic history. At the autopsy all the arteries of both the aortic and pulmonary systems were found to be diseased. There was consolidation with infarctions of portions of both lungs. Both renal arteries were occluded by thrombi. There were small clots in each of the lateral lobes of the cerebellum. The left ventricle was dilated and hypertrophied. For a distance of three inches above the bifurcation the aorta was so narrow as scarcely to admit a bullet-probe. The arterial wall measured only one-half inch in breadth when opened. The kidneys microscopically showed no signs of nephritis. The arteries, macroscopically, showed gray translucent spots and patches, smooth or corrugated. Microscopically, these were composed of indistinct fibrocellular tissue in the intima. In the media and adventitia were collections of leucocytes, especially around the vessels. The new tissue was not the seat of fatty or degenerative change, with the exception of some deeply-stained patches of amorphous material (lime?) on the surface.

(2) The hæmorrhages in our case caused prominent symptoms during life. Those in the lungs were easily made out. The intra-splenic hæmorrhage was diagnosed from the acute enlargement and tenderness of the spleen and the rise in temperature. The extravasation in the mesentery was indicated by excessive abdominal pain, bloody stools, and tympanites; and the sudden occurrence of sub-

* *Transactions of the London Pathological Society*, 1892, p. 46, cited in part by Delafield (4).

normal temperature indicated a large hæmorrhage. The question whether the hæmorrhages were due to thromboses, embolisms or ruptures of smaller or larger vessels had to be left open as any one of these conditions was possible. The marked bleeding from the gums, however, indicated that it was not necessary to assume the existence of embolism or thrombosis and that the hæmorrhages might be explained by the existence of a hæmorrhagic diathesis. Such a diathesis in similar cases has been noted by Filatoff (8) and Förster (11).

(3) The arterial changes present were very advanced. The question naturally arises whether these or the renal changes were primary. This is frequently a very difficult problem. From the examination of two other members of the same family, we are inclined to believe that the lesion in the kidney was primary, as these two persons have long-standing chronic nephritis with but little evident arterial change. Of course, it is possible that their peripheral arteries may not show arteritis, while this may exist elsewhere, and, therefore, a positive opinion is not warranted.

Throughout the records, there are occasional references to cases of chronic arteritis in children, and it has been noted that there exists in certain families a tendency to the development of arterial changes early in life. The most common occurrences have been aneurisms of the cardiac valves and dilatation of the aorta (Baginsky). Jacobi, (18), in reporting a case of aneurism of the abdominal aorta in a child, refers to twenty-eight other recorded cases of aneurism in early life. Since then at least two more cases have been described. These cases need classification, however, as some of them were not due to primary arterial changes but were mycotic in origin (Eppinger). Among the striking instances of advanced arterial disease reported in childhood, are the following:

Fenomenoff has recently reported a congenital aneurism of the abdominal aorta which obstructed delivery (Eichhorst). Oppe (19) describes an aneurism of the basilar artery in a boy seven years old. Sanné (20) reports the case of a child of two years with narrowing of the aorta due to chronic aortitis. Pendin (21) describes in a girl of 12 an aneurism of traumatic origin. Andral (22) saw calcific plates in the aorta of

a girl 5 years old. Moutard-Martin (23) saw atheroma of the arch in a boy aged two years. Hodgson (24) records calcification of the temporal artery in a girl of five, and Hoffmung (25) describes an aneurism of the pulmonary artery in a girl of ten months. Generalized arterial disease in connection with chronic interstitial nephritis without any syphilitic history has been described by Filatoff (8) and Barlow (6).

The changes found in our case were as advanced as in well-marked arteriosclerosis of adult life. While the character of the lesions suggested syphilis, a very thorough examination of the whole family failed to elicit anything corroborative of such a view.

(4) The absence of any usual etiological factor drew our attention to the possibility of the case being an instance of so-called "family nephritis." Senator's recent volume on the diseases of the kidneys, in Nothnagel's Handbuch, cites several instances of nephritis occurring in several members of the same family.

Dickinson (26) reports a family in which in the first generation two sisters had albuminuria for many years and died at the ages of 48 and 49. Of the four children of a brother, one son had albuminuria for 14 years and died at the age of 26, and one daughter died at 34, after having been sick sixteen years. Two other daughters had no albuminuria and lived to the ages of 38 and 40. Of six children in the third generation, five had albuminuria, one daughter having it, from the age of nine months, for twenty years, and one son of 20 for an unknown length of time. Another son of 15 had it for two years and one daughter of five years was albuminuric since the age of six months.

Tyson (27) mentions the instance of a man of 30 with contracted kidneys whose father and mother died of the disease, and one brother at the age of 37. Two children of the brother had chronic nephritis at the ages of four and seven respectively. One brother died at 29 from convulsions. Two older brothers and one sister, aged 23, 32 and 36 respectively, had the disease for five or six years. One cousin on the mother's side and numerous relatives in earlier generations died of chronic nephritis.

Eichhorst (28) gives the history of an artist's family who were sufferers from the disease. The grandmother (who had no gout) died of uræmia. The mother and a daughter 24 years old suffered from chronic interstitial nephritis, the former for fifteen years. Two sons died of

uræmia. A daughter, aged 22, showed signs of the disease at the time of the report.

Kidd (17) reports the case of a woman of 60 who died of nephritis of long standing, as did also her brothers. Of 12 children, 7 died with the same disease, and two had it at the time of his report. In two of the fatal cases a post-mortem examination was made and contracted kidneys were found. Possibly Förster's cases belong in this category.

Samelsohn (29) observed chronic nephritis in two brothers, and noted its probable existence in two sisters and a son in the same family.

Pel (30), in a recent article, mentions several families in which he has noted the tendency to the development of chronic nephritis, and reports one instance of family nephritis not less striking than that of Dickinson. The disease affected 18 members (9 males and 9 females) of the family in three generations. Of 60 children of the fourth generation, none at the time of Pel's report was affected, but as, in contrast with the observations cited above, the disease in this series appeared later in life, their subsequent fate must remain for the present in doubt. Pel notes that in this family the tendency was for the sons to inherit from the father and the daughters from the mother—an exception to the Darwinian rule of heredity.

An examination into the family history of our case and a personal investigation of the members of the family, which was possible only after repeated efforts, revealed the following interesting facts:

The father died at the age of 42 of lobar pneumonia. His previous history was negative so far as can be determined. The mother is 45 years old and in good health, presenting no signs of renal or arterial disease. Of the 12 children, six died very young of causes which are unknown. The living children are as follows:

1. A girl of eight, who seems to be in good health and whose urine and heart show no changes.

2. A boy, aged 13, who is also in good health and in whom the physical and urinary examinations reveal nothing abnormal.

3. A girl of fourteen, who complains of severe headache. She is very well developed and her heart and arteries show no deviation from the normal. The urine has a specific gravity of 1016 and contains no albumin nor casts.

4. A girl, aged 19, who has been ailing for at least six years (probably over 10 years). She has no history of any acute disease. Menstruation be-

gan at 12 and has always been irregular and profuse. She complains of very great thirst and has to arise several times at night to drink water. She passes very much urine. The appetite is poor. She has no headache and no vomiting. She has occasional cough and palpitation, but complains only of her excessive thirst. An examination on November 5, 1898, showed the following: There is marked anæmia. The pulse is of high tension and the walls of the radials are somewhat thickened. The sphygmographic tracing shows marked elastic vibrations and only a slight dicrotic notch. The apex beat is in the fifth space just to the left of the mammillary line and is very forcible. The urine is very light in color and has a specific gravity of 1001½. There is albumin present, 0.14 per cent (Esbach), no sugar. The microscopical examination shows only epithelium and a few leucocytes. When seen two weeks later, there was marked œdema of the eyelids and some headache, which disappeared after a few days. At the date of writing, March 1, 1899, she is in good health, except for her inordinate thirst. She is evidently suffering from a very advanced chronic interstitial nephritis, which is running a slow and quite latent course.

5. The oldest son, aged 24, gives no history of any acute disease in childhood. He has always been anæmic. Six years ago, he suffered all winter from a severe cough and had general œdema which began in the face. He has felt perfectly well since then and, therefore, for a long time opposed any personal examination. On February 19, 1899, the following facts were made out. There is a moderate anæmia. The apex beat is in the sixth space, one-half inch to the left of the mamillary line, and is very forcible. There is a booming first sound and an accentuated second sound at the apex. At the aortic orifice, there is a slight systolic murmur and an accentuated second. The pulse is 86, the radial artery is slightly thickened, the pulse shows moderate tension. The urine is clear, 1012; contains no albumin and no casts: urea 9 grains to the ounce. Although he has no symptoms at present, and the urine shows nothing abnormal, there can be little doubt from his history and physical examination, that he has a chronic interstitial nephritis and belongs to that unusual class of cases in which for a time at least the urine shows no abnormalities. We have been permitted thus far to examine only one specimen of the urine, and it is quite possible that when we next examine it we may find albumin.

It is evident that we have to deal with a family in which there is a tendency to the development of chronic interstitial nephritis, this having occurred in the three eldest children.

The main points of interest in the case herewith presented are as follows:

1. The occurrence of a very advanced primary chronic interstitial nephritis at the age of fourteen years.

2. Its presence in other members of the same family.

3. The extensive and marked arterial changes present.

4. The hæmorrhagic diathesis and especially the occurrence of a large hæmorrhage in the mesentery.

5. The occurrence of calcific deposits in the liver.

6. The case draws our attention again to the latency of some of these cases of chronic nephritis in children. There is no doubt that some of them have been regarded as instances of diabetes insipidus. The necessity of a careful and continued observation of the heart and vessels in such cases is apparent. Others are treated for a long time for anæmia without its cause being discovered. Still others do not present themselves for treatment until the fatal termination is close at hand, the patients having had no marked symptoms. The fact that chronic nephritis may run so latent a course and may occur at any age should lead us to pay as much attention to the examination of the urine of children as of adults.

We are indebted to Dr. F. S. Mandlebaum, the pathologist of Mount Sinai Hospital, for much valuable assistance in connection with the histological part of this investigation.

DESCRIPTION OF PLATE XXV.

Fig. 1.—Section of liver showing areas of calcification. Low magnification.

Fig. 2.—Section of kidney showing obliterating endarteritis, calcified vessel, and chronic interstitial nephritis. Low power.

Fig. 3. Section of calcified artery seen in Fig. 2, with higher magnification.

Fig. 4.—Section of kidney showing the lesions of advanced chronic interstitial nephritis.

Fig. 5.—Section of wall of calcified coronary artery of the heart.

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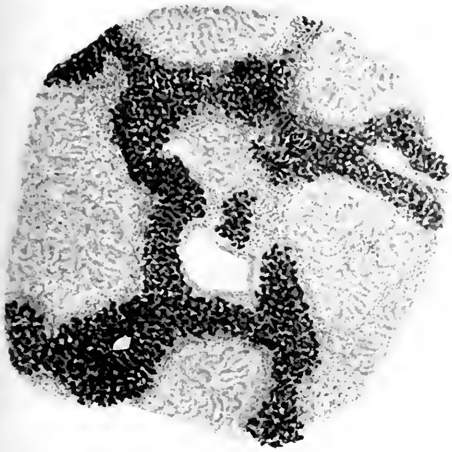

FIG. 1.

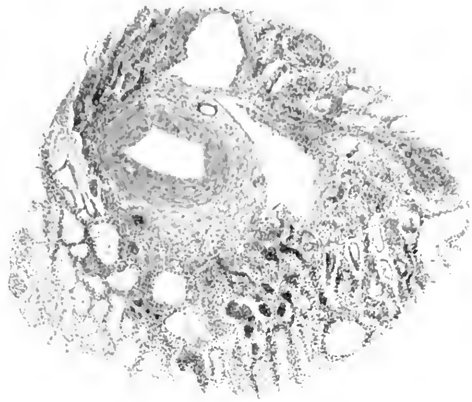

FIG. 2.

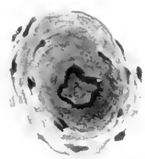

FIG. 3.

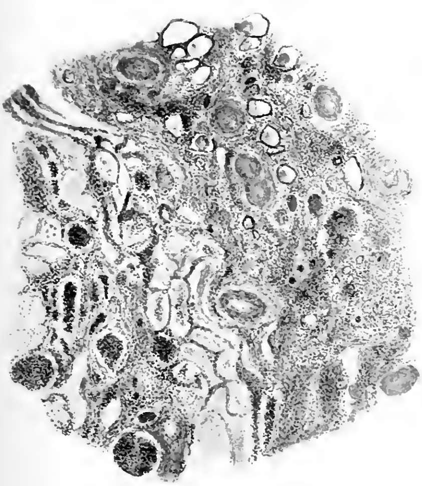

FIG. 4.

FIG. 5.

LYMPHOMA, A BENIGN TUMOR REPRESENTING A LYMPH GLAND IN STRUCTURE.

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Plates XXVI and XXVII.

There is little uniformity of opinion among authorities to satisfy the critical student concerning the word "lymphoma" and the conception conveyed by it. In conformity with oncological nomenclature, the term is, in a strict sense, applicable only to new growths made up of lymphoid tissue, but in the course of time it has come to include a variety of pathological conditions.

Kaufmann (1) and Orth (2) classify lymphomata into leukæmic, aleukæmic and malignant. Klebs (3), following Virchow, Birch-Hirschfeld (4) and Ziegler (5), emphasize the fact that in many instances inflammatory processes have monopolized the use of the term. Langhans (6) many years ago stated that "in pseudo-leukæmia there are two forms of lymphomata;" and Warren (7), in enumerating the conditions denoted by the word "lymphoma," mentions "simple hypertrophy of the lymphatic glands, due to some of the above causes (tuberculosis, syphilis and other infective diseases), to which the term lymphoma has been applied." Warren advocates dropping the term lymphoma, since "those cases which were supposed to occupy an independent position under the name of lymphoma or lymphadenoma can now be classified under some one of the other headings." The objection to this by Birch-Hirschfeld is that if the term lymphoma is limited to the infectious enlargements of lymph glands to which it has so far been mainly applied, there remains nothing by which to designate a benign lymphomatous tumor.

Most unsatisfactory is the definition of Stengel (8), who defines lymphoma as "a more or less malignant form of new growth affecting

the lymph glands or other lymphadenoid tissues." Unna (9) evidently considers a lymphoma to be simply a collection of leucocytes, for he says, "we shall not go far astray if we regard this very obstinate form of leukæmic cutaneous nodule also as a granuloma and not as a collection of leucocytes—a lymphoma."

With gratifying clearness Senn (10) defines lymphoma as "a benign tumor formed of lymphoid tissue produced from a matrix of lymphoblasts;" further (11), as "an encapsulated tumor which manifests no tendency to implicate adjacent glands and which is never complicated by affections of the blood-forming organs." That this conception of a lymphoma was largely theoretical in its deduction is evidenced by the admission of the author that "it is easier to say what a lymphoma is not, than what it is." The reason for this last statement obviously lies in the absence from medical literature of any observations of growths which would conform to such a definition, *i. e.* a true tumor, benign, atypical in structure, yet representing lymphoid tissue and not due to any inflammatory process. Not even Senn refers to any such observations; he remarks further, "In no department of surgical pathology do we meet with more confusion than in the difference between benign and malignant tumors and inflammatory swellings of the lymph glands." This confusion is due not so much to a lack of knowledge of the inflammatory affections of lymph glands nor to an ignorance of the nature and characteristics of malignant tumors but to the great rarity of benign new growths of lymph glands.

I am indebted to Dr. J. B. Murphy for the opportunity to examine a small tumor, which, I became convinced from a careful study, represented a benign, non-inflammatory, new-growth of lymph nodes; in other words, a lymphoma according to Senn, a benign lymphoma according to Ziegler and others.

The tumor was located in the left groin and had attained the size of a pigeon's egg by a very gradual growth extending over a period of fifteen years. The inguinal canal was not patent and there was no enlargement of the adjacent glands nor of those of the opposite side. The man was in good general health; there were no similar

tumors elsewhere on the body, and there has been no recurrence of any growth since its removal three years ago.

When examined in the fresh condition the tumor presented an oval, flattened shape not very unlike a small kidney (Plate XXVI, Fig. 1). On section, light reddish surfaces were exposed which were studded with areas the size of millet seeds and resembling closely the Malpighian bodies of the spleen. These minute areas were very uniform in their distribution; there was apparently no difference in this respect between the cortical and the more central portions, these two regions being quite similar in color. Although a fibrous capsule surrounded the growth, there were no coarse trabeculæ entering the tissue. The blood-vessels exposed in a number of sections were all small. There were no spots of softening or hæmorrhage nor any special features which would point to an inflammatory process.

Microscopic Examination.—The tumor was hardened in alcohol. Sections stained by ordinary methods show the tissue to be distinctly lymphoid in character and to contain many lymph nodes (Plate XXVI, Fig. 2). In serial sections the largest diameters of one of average size were 112 by 88μ. A very large node measured 160μ in its longer diameter by 116μ in the shorter. The cells composing these nodes are arranged in a very striking and peculiar manner. Around the periphery they form encircling rows which extend for one-third or, in rare instances, one-half of the circumference (Plate XXVII, Fig. 4). The arrangement in rows is more perfect in some nodes than in others, but when serial sections are examined it is found to occur at some point and to some degree in every node. Frequently these rows are double: or three or four rows form a band, which, extending around the node for some distance, breaks up to anastomose with similar bands or single rows. Between these rows and bands are cleft-like spaces (Plate XXVII, Fig. 4) which are more marked in some preparations than in others, and in some are undoubtedly to be ascribed to shrinkage. The rows are most perfect at the periphery, so that the edge of the node is usually well defined. This is, however, not invariably the case, for some nodes fuse gradually with the internodal tissue, while short tangential rows shoot out into the surrounding

tissue, obscuring the distinctness of the margin. The cells themselves which form these rows are small, 4 to 5 μ in diameter, with a nucleus which occupies almost the entire cell, leaving only a narrow margin of cytoplasm (Plate XXVII, Fig. 6). The nuclei are round, with a deeply staining nuclear membrane. The chromatin frequently forms an irregular lining for the nuclear membrane and usually two or three centrally located large granules.

The reticulum supporting these cells is very scanty in the outer zones of the node. Here it forms delicate strands which extend between and parallel to the rows. Toward the centre it is more dense and forms heavier bands. It is always greater in amount and, in carefully pencilled preparations, is found to produce a finer meshed network around the blood-vessels.

Blood-vessels can be found in every node by the study of serial sections, and there are generally one or two in the majority of the sections from any one node. The vessel enters obliquely and frequently divides after its entrance. In such cases its further course is not easily followed. These vessels are evidently arterioles; they possess thick hyaline walls. In case the blood-vessel is cut directly across, the row formation is very perfect; row after row or layer upon layer surround the vessel throughout the entire nodule. When the vessel is cut obliquely the row arrangement is more regular at the periphery, the remainder of the node consisting of the same small darkly staining cells grouped in a dense mass and devoid of any formation of rows. Figure 3 of Plate XXVI shows the rows of lymphoblasts about one of the larger internodal blood-vessels.

Each node is found to possess at some point a group of cells which afford a marked contrast to those just considered. This group is generally central in its location but in some sections is nearer to one pole of the node (Plate XXVI, Fig. 2, and Plate XXVII, Fig. 4). The cells forming these groups are large, 8 to 10 μ in their long diameter, and like the others consist mainly of nuclei. But these nuclei are oblong and irregular or notched and stain faintly with the exception of the nuclear membrane. In examining successive sections one reaches the conclusion that these groups of pale cells occur at or near

the point where the arteriole breaks up to form capillaries, and, further, that the individual cells resemble in size, shape and staining those which line the blood-vessels. The division into capillaries results in a denser reticulum which, with the large faintly staining cells, forms an area differing very much from the densely packed, darkly stained cells which surround it. When, as exceptionally is the case, a rather large arteriole passes into a node and immediately divides into a number of small branches, the resulting pale centre is quite large.

Thus it is seen that the light areas resembling the Malpighian corpuscles of the spleen, observed when the tumor was first cut, are lymph nodes which are remarkable for their extraordinary size and the extréme regularity in the arrangement of the cells forming them; also that these nodes are formed around blood-vessels or at points where the blood-vessels divide to form capillaries.

The internodal tissue is loose and shows considerable stroma, which supports small cells similar to the darkly staining cells of the nodes. There is no particular arrangement of these except that they are more numerous at the edges of the spaces formed by the reticulum, the centres of such spaces being empty. The reticulum of this internodal tissue, stained by Van Gieson's stain (Plate XXVII, Fig. 5), picronigrosin or the iron-hæmatoxylin method and in carefully pencilled sections, shows no nuclei which can be positively said to be a part of the stroma in spite of the fact that the nuclei of the cells lining the spaces often appear as though they belong to the reticulum, as though in fact the reticulum were connected with cells. The blood-vessels in the internodal tissue are larger than any found within the nodes. The arteries possess thick hyaline walls with two, three or more elastic sheaths and occasional isolated elastic fibres (Plate XXVII, Fig. 7). The thickening in the walls of the arteries and arterioles has affected only the tunica media. As the vessel becomes smaller its wall becomes more and more homogeneous and hyaline in character, until finally no nuclei can be seen in its middle coat. The veins are easily recognized by their thin walls; they show no changes. Blood is quite generally absent from the vessels, a small amount occurring in the

largest. There are no heavy bands of stroma which extend in from the capsule. The capsule itself is thin, largely fibrous, with few nuclei.

The similarity of the nodes, which form the greater and more interesting part of this tumor, to the "secondary nodules" of lymphoid tissue described by His, Flemming and others is very apparent.

Hoyer (12), in describing the cells in lymph glands, referring to the pale centres of the germinal areas, speaks of "the cells described by Flemming with large nuclei which give the centres of the germinal areas their pale color."

Hoehl (13), in a good description of the reticulum of lymph glands, states that the connective tissue surrounding the germinal areas is arranged in a tangential direction as regards the periphery of the nodes, and Flemming (14) says that in the germinal areas it must be that there is a form of slow centrifugal pressure whereby the younger cells are pressed toward the periphery and driven out through the spaces of the reticulum. It can be seen from these citations and from the structure of the tumor that cells were produced in these pale centres and pressed toward the periphery, but lacking a normal exit through lymph sinuses into the circulating blood were accumulated in the growth and produced not only a remarkable formation of rows around such germinal centres, but also extremely large nodes. Concerning the formation of lymph nodes about blood-vessels, Flemming (15) finds that "the view that the arrangement of the lymph node about the blood-vessel originates in the germinal area, is perfectly acceptable." Also Ribbert (16) has noted that lymphomata occur on blood-vessels without any increase in the number of leucocytes in the blood.

Hansemann (17), in a consideration of the tumors which may develop from lymph glands, specifies three varieties of cells from which they may originate, viz.: the lymphocytes which form the parenchyma proper, the endothelial cells and the cells which form the framework (Gitterzellen). The existence of the last is positively denied by Hoehl (18), who says "the reticulum is entirely devoid of nuclei."

Senn (19) tells us that in true lymphoma "the follicular structure

(nodes) is well preserved," and that "the lymphoblasts produce lymph corpuscles which are not transformed into leucocytes but remain in the reticulum of the tumor as the essential cell elements."

It is well to note the resemblance in structure of the nodes in this tumor to the corpuscles of the spleen, for Albrecht (20) has reported a case in which numerous dislocated spleens were scattered over the peritoneal surfaces. Their number was estimated at about four hundred, and they varied in size from those which were strictly microscopic to some the size of a walnut. The greater number were found in the upper left quadrant of the abdominal cavity and on the posterior surface of the greater omentum. But they were found also in Douglas's pouch and on the upper one-third of the rectum. Eight were examined microscopically and found to resemble splenic tissue in every respect. They contained large quantities of blood and blood pigment, and in one of the larger examples typical splenic follicles were found about the blood-vessels.

The absence of blood pigment, as well as the scanty amount of blood and the lack of coarse trabeculæ would, in themselves, be sufficient to prevent a conclusion that the above-described growth represents or has sprung from displaced splenic tissue. The pale germinal areas present in the nodes of the lymphoma and absent from splenic follicles afford another striking difference. Albrecht also found accumulations of round cells about the masses of blood pigment, whereas the absence of any of the histological features of inflammation in this growth constitutes one of the main reasons for concluding that it is a true lymphoma.

Freund (21) has called attention to the resemblance between the accumulations of cells around the blood-vessels in periarteritis nodosa and lymphomata as follows: "Indeed one sees not seldom circumscribed areas of cells in the adventitia which have some similarity to lymphomata." In periarteritis nodosa, however, many vessels of the body and in different sites are the seat of such accumulations. The clinical picture is often that of a toxic neuritis or a toxic myositis and not only is the process in and around the vessel wall histologically an inflammatory one, but there are also changes in the organs due to obstruction of the vessels.

In concluding, this tumor seems to form a veritable confirmation of the words of Lancereaux (22), who in 1875 wrote: "It is indeed probable that this lymphoid tissue gives birth to young cells, and one can see how, under the influence of a mild irritant, the production of these cells becomes more abundant; the elements of the network multiplying at the same time, it results that tumors occur which have for their common characteristics a reticulated network in which the lymphoid elements are accumulated more or less abundantly. Such tumors or lymphatic growths are designated by the name—lymphoma."

DESCRIPTION OF PLATES XXVI AND XXVII.

PLATE XXVI.

Fig. 1. Photograph of lymphoma (exact size). The tumor has been cut open and from the right half portions have been removed for examination.

Fig. 2. Photograph of section showing lymph-nodes and internodal tissue. Low power.

Fig. 3. Formation of lymphoblasts about one of the larger internodal blood-vessels.

PLATE XXVII.

Fig. 4. Photograph of centre of a node showing the pale germinal area.

Fig. 5. Reticulum of the internodal tissue. Pencilled section; Van Gieson's stain.

Fig. 6. Cells of the rows of lymphoid tissue.

Fig. 7. Cross-section of one of the larger internodal vessels showing the elastic sheaths. Orcein stain.

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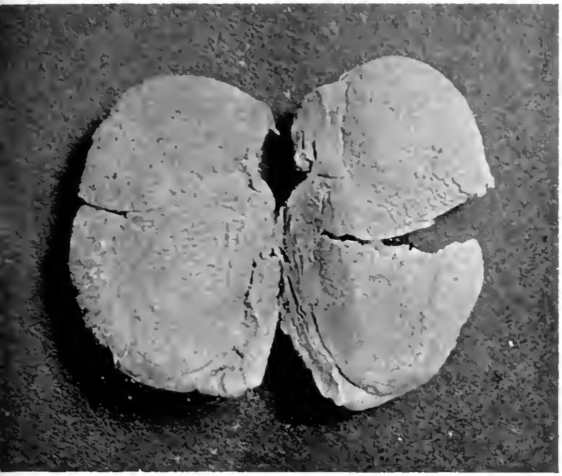

FIG. 1.

FIG. 3.

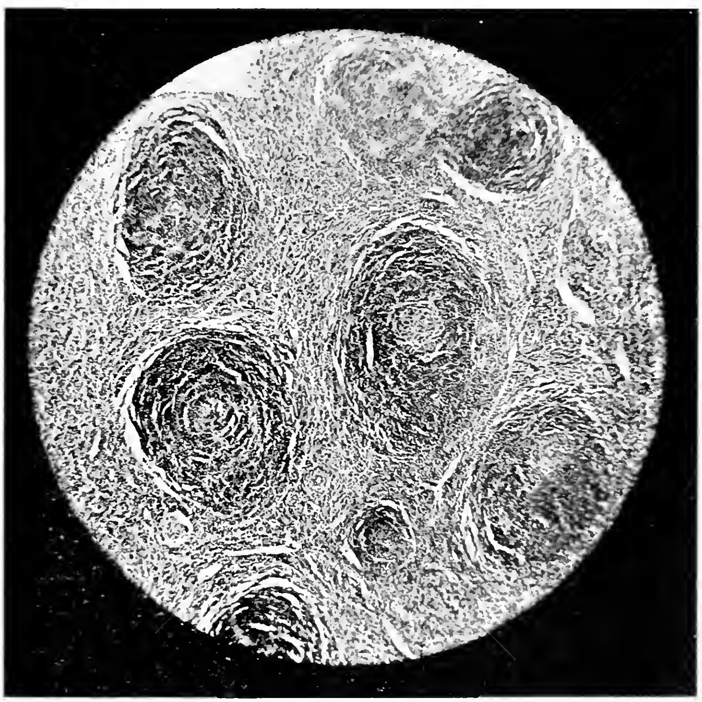

FIG. 2.

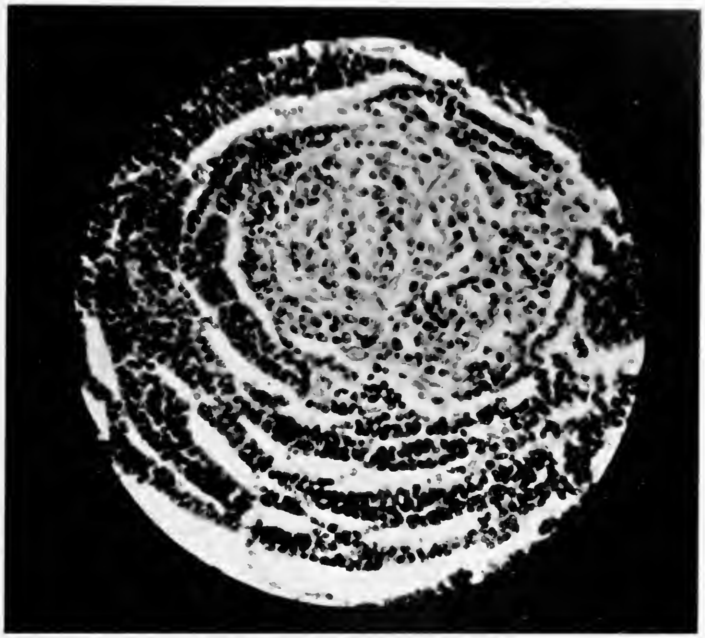

FIG. 4.

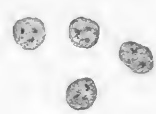

FIG. 6.

FIG. 5.

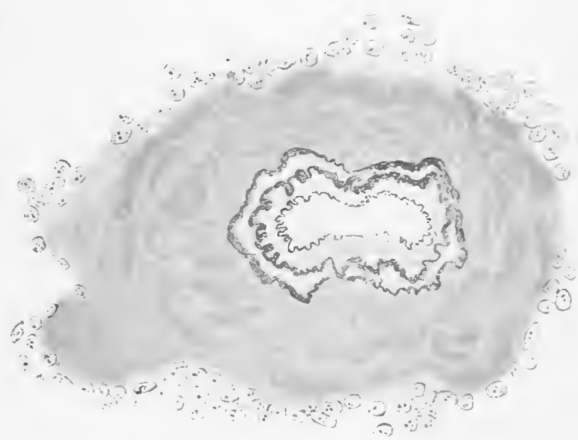

FIG. 7.

OBSERVATIONS CONCERNING LEUKÆMIC LESIONS OF THE SKIN.

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Some time ago I published in the *Yale Medical Journal** a preliminary report of a case of leukæmia cutis. I present now a more extensive study of this case. For the sake of convenience much of the matter of the previous article will be included here.

The rarity of leukæmic cutaneous affections may be inferred from the fact that a number of text-books on medicine and even on dermatology either do not speak of this condition at all, or simply mention it in a general way. In Unna's book† on the pathology of skin diseases there may be found a critical summary of 9 cases, and while perhaps a few may have escaped detection, probably scarcely more than a dozen cases are on record. Kaposi's case (1885) is the most frequently cited, and has directed especial attention to these lesions: the first observation, however, goes back to Biesiadecki's report in 1876, whose article is often quoted also with reference to the theory of leukæmia. The leukæmic affections of the skin which have hitherto been observed may be grouped into three classes.‡

The first class is represented by cases in which there are circumscribed, multiple, pinhead to hazelnut-sized, rapidly growing, pale or faintly red to brownish-colored tumors, irregularly distributed over the body, with little tendency to retrograde metamorphosis or ulceration. To this group belong the cases of Biesiadecki, Hochsinger and Schiff, Oliver, and Philippert. Hochsinger and Schiff found in their

* January, 1899.

† Unna, Die Histopathologie der Hautkrankheiten. Berlin. 1894.

‡ In this classification I depend upon Unna's presentation of the subject. I therefore omit the bibliography contained in his book, *op. cit.*, p. 619.

case cellular infiltrations, situated especially between cutis and hypoderm, which had changed the superficial fat layers to lymphoid tissue, and closely surrounded the glands. The infiltration started from the capillaries of the coiled tubes and the superficial fat layers, and consisted of lymphoid cells without spindle or giant cells. The upper part of the cutis and the papillary portion were almost free from infiltration except where, by constriction from above, the cutis would meet the infiltration. The nodule did not contain any blood-vessels; at the periphery these were dilated, being distended with blood. The coiled tubes were well preserved within the nodule. In Oliver's case there were 60 subcutaneous nodules presenting the structure of a round-celled sarcoma. Philippert observed subcutaneous, brownish-colored, smooth tumors on the face and head, appearing later in the nasal and pharyngeal mucous membranes. They possessed an adenoid structure. In a case of acute leukæmia described by Seelig,* the skin behind the ears and in different situations over the thorax and abdomen showed, three days before death, small, freely movable nodules, and small hæmorrhages. Microscopically the nodules were composed of lymphoid tissue, embedded in the subcutaneous fat, and containing small lymphocytes and larger uninuclear cells, the whole surrounded by a delicate connective tissue capsule.

The second class is characterized by a few, solitary, brown, markedly elevated, lobulated, firm, slowly growing, and persistent nodules. It is illustrated by Neuberger's case, according to whom the condition consists of a small lymphoid-celled infiltration into the cutis, especially around the follicles, by which the deeper layer of the epidermis is compressed, although it is still separated from the infiltration in the lower part of the cutis by a small, narrow, non-infiltrated zone of connective tissue. The surroundings of the coiled tubes, the hypoderm, and the muscular tissue of the cheeks were also infiltrated. Neuberger regards the coiled tubes, which themselves remain intact, as the starting point. Around them the lymphomatous formation appeared more distinct than in the upper parts of the cutis.

The third class, finally, is represented by cases of which that of

* *Deutsches Arch. f. klin. Med.*, 1895, liv, 537.

Kaposi may serve as a type. It is a diffuse form, showing on the one hand a diffuse, moist, eczematous appearance, especially of the head, extremities and chest, and on the other hand tumors the size of a pea to a pigeon's egg, which ulcerate spontaneously, leaving a large, flat, red ulcer. An œdematous condition of the skin is present. Sections in Kaposi's case showed a marked œdema of the hypoderm, which could be distinctly differentiated from the healthy yellow adipose tissue. The interstitial tissue between the fat lobules had sclerosed and traversed the œdematous masses in the form of thick white septa. The œdematous could not be distinctly separated from the healthy parts surrounding them. The cutis above the nodule was œdematous, or reddened and ulcerated, or confluent with the nodule and of an amyloid appearance. Microscopically these tumors consisted of a non-vascular, fine stroma, with dense leucocytic infiltrations, most prominent in the region of the coiled tubes. The hair follicles were unchanged. The rete Malpighii showed partly a proliferation, partly a rarefaction, produced by the approaching infiltration. Galliard's earlier observed case is very similar. He found a reticulated formation, pronounced around the sweat and sebaceous glands, and between the fat lobules. In the more recent nodules the superficial layers were relatively undisturbed. In Leber's, and Chavel's cases the eyelids especially were the seat of a diffuse infiltration. Here also the microscopic examination revealed small cells in a fine reticulated stroma.

In a patient suffering from lympho-lienal leukæmia, exhibited by Litten* to the Berliner medicinische Gesellschaft, the upper eyelids presented a swollen appearance suggestive of œdema, but which closer examination showed to be due to small lymphatic tumors, over which the skin was freely movable. No microscopical examination was reported. In the discussion Köbner commented upon the extreme rarity of leukæmic affections of the skin and remarked that lymphomata, which may cause a similar condition, extend, in contrast with leukæmic tumors, into the subconjunctival tissue.

Kelsch and Vaillard's† case of multiple, metastasizing, malignant

* *Deutsche med. Wochenschr.*, 1897, Vereins-Beil., i, p. 3.

† *Ann. d. l'Inst. Pasteur*, 1890, iv, 276.

lymphomata, appearing in the skin near the eyes, and also in the thyroid, abdomen and bones, although interpreted by them as leukæmic, was not, as pointed out by Schmorl,* demonstrated to be an instance of leukæmia, and probably does not belong to the class of affections now under consideration.

From the foregoing it appears that the leukæmic dermatoses have in common a small-cellular circumscribed or diffuse infiltration, which originates in the deeper layers of the skin and extends upward.

Little attention, however, has been given to the more detailed study of the characters of the cells making up this infiltration. While the formation is regarded by most observers as a lymphoma, this idea is apparently based more upon the general characters of the case than upon any definite investigation of the tumor. The term "small-celled infiltration" is an exceedingly indefinite and wide one, applying to pathologically entirely different conditions, so that, whenever it is possible, more definite and precise descriptions should be given. It is for this reason that Unna doubts that these structures really represent lymphomata. He is inclined to regard them as granulomata, containing plasma cells, especially as he believes that they present in their circumscribed foci the characteristics of such growths.

The nature of leukæmic affections of the skin has been recently discussed with much fulness by Nékám,† who reports a typical case, but before considering his views it will be well to state the opinions held concerning the characters of the secondary leukæmic nodules which are observed with much greater frequency in other organs of the body, especially the liver, than in the skin.

Virchow‡ regards the leukæmic nodules, which may appear in the liver, kidneys and other situations normally devoid of lymphatic tissue, as true neoplasms. He points out that the new formation may appear in the form either of an infiltration or of definite nodules and says: "A kind of new lymph-glands may thus be developed within an organ which otherwise contains nothing of the sort. . . . We possess

* *Centralb. f. allg. Path. u. path. Anat.*, 1891, ii, 118.

† Die leukäm. Erkrankungen der Haut. *Monatschr. f. prakt. Dermatologie*, Ergänzungsheft, 1899, ii.

‡ Die krankhaften Geschwülste, ii, p. 568 et seq. Berlin, 1865.

in leukæmia a very complete picture of the gradual generalization of an originally local process, in which we can trace the individual steps more definitely than in any other kind of generalization."

A similar interpretation of leukæmic nodules as the seat of active formation of new cells is advanced by Bizzozero,* who differs from Virchow, however, in regarding emigration from the blood as the primary source of the leucocytes in these secondary nodules. But Bizzozero does not find that the nodules are merely passive accumulations of migrated cells, for he was able to demonstrate not only in the hyperplastic spleen, lymphatic glands and intestinal follicles, but also in the secondary nodules in the liver and kidney, abundant karyokinetic figures within the lymphoid cells. This abundance of nuclear figures is, according to Bizzozero, one of the marks distinguishing these leukæmic nodules from the accumulation of leucocytes in suppurative foci.

Similar observations as to the occurrence of cell proliferation in leukæmic nodules in internal organs have been made by M. B. Schmidt† and by Hindenburg,‡ both of whom note the presence of indirect cell division also in the capillary endothelium, particularly in the liver. These writers, therefore, agree with Virchow's conception as modified by Bizzozero.

In contrast to the preceding views is that held by Rindfleisch,§ Cornil and Ranvier|| and Löwit,¶ who regard the secondary leukæmic nodules and infiltrations as merely passive accumulations of leucocytes derived by migration from the blood. Ziegler** attributes the nodules to migration of cells from the blood followed by local proliferation of cells in the foci. Klebs†† places leukæmic nodules under the head of his leukocytomata and allies them with tubercle, leproma and other infectious granulomata.

* Virchow's *Archiv*, 1885, xcix, 378.
† Ziegler's *Beiträge*, 1892, xi, p. 228 et seq.
‡ *Deutsches Arch. f. klin. Med.*, 1895, liv, 209.
§ Lehrb. d. path. Gewebelehre, Leipzig, 1886, p. 497.
|| Manuel d'histologie pathologique, i, p. 296. Paris. 1881.
¶ *Situngsb. d. k. Akad. d. Wissensch.* (Wien), 1886, xcii.
** Lehrb. d. spec. path. Anat., p. 9. Jena. 1898.
†† Allgem. Pathologie, ii, p. 597. Jena. 1889.

The most precise statements hitherto recorded concerning the composition of leukæmic cutaneous nodules are to be found in Nékám's* recent discussion of the subject. A critical review of the records has convinced him that a number of cases reported as examples of leukæmia cutis, and especially the diffuse lesions falling under the third group (lymphodermia perniciosa), cannot properly be regarded as such inasmuch as neither the clinical nor the anatomical characters of leukæmia or of the tumors were indisputably established. He accepts as undoubted examples the cases of only Biesiadecki, Hochsinger and Schiff, Neuberger, and himself, in all of which there were definite nodules the size of a pea to a hazelnut without a diffuse appearance. Nékám thus describes the process: "The condition starts with an œdema of the skin, followed by a diapedesis of all the constituents of the blood along the course of the larger blood-vessels. This infiltration appears rapidly in the tissues surrounding the vessels of the coiled tubes, the superficial fat layers, the cutaneous muscles, and the follicles, and extends in an anatomical direction, but in a somewhat irregular manner, diffusely, or in streaks, or in lymphoid masses. The cells are chiefly lymphocytes, very infrequently plasma cells. Mitoses, giant cells, newly-formed blood-vessels, and a change of lymphocytes into plasma cells could not be seen. The cells in the centres of the nodules persist unchanged. Now, inasmuch as in all probability the process of emigration of cells from the blood is a continuous one, whereas the growth of the nodules is very slow, attaining, even in years, only small dimensions, it follows that cells must disappear from the nodules. In support of this view is the fact that red corpuscles and eosinophilic cells are found only in recent nodules. . . . The red corpuscles are removed by the lymphatic vessels. Other cells are destroyed in the subpapillary layers and their granular fragments appear in the rete Malpighii and on the surface of the skin. This may be regarded as a useful elimination of leucocytes. . . . These observations make it evident that leukæmic tumors of the skin must be regarded as infiltrations, and we can, therefore, accept as such only those which fulfil the following conditions: (1) They must

* *Loc. cit.*

occur during the course of true leukæmia; (2) their origin must be exclusively a diapedesis of cells from the blood, local proliferation of cells being absent; (3) a part of the cells, especially the red corpuscles, are returned to the blood by way of the lymph-channels, another part, especially the leucocytes, are carried off through the epidermis, or otherwise discharged; (4) the chief bulk of the cells composing the nodules undergo no metamorphosis. The atrophy of the normal connective tissue leads to the formation of a rarefied network in which the lymphoid cells lie embedded, and this adenoid character of the tissue persists for years. The infiltration starts around the deeper vessels and extends rapidly upwards into the papillary layer." *

For the study of the case which I here report I was not able to make any prolonged observations during the life of the patient and I encountered difficulties in obtaining sufficient material for as thorough histological investigation as I desired.

History.—The patient was a man of about 40 years, unmarried, who had been in fairly good health until about two years before, when he was first taken ill with "various digestive disturbances and malaria." The attending physician, who treated him from that time on until his death, states that at that time he had an irregular intermittent fever and an enlarged spleen, which he regarded as an amyloid (?) spleen consequent to malaria. This "malaria," which finally subsided, was characterized by a stubborn resistance to quinine, and arsenic was, therefore, given in addition. The patient, however, recovered sufficiently to attend to his duties as a clerk. His appetite was of the best, and indeed continued so until two days before death, when violent vomiting set in. Several months (how many cannot now be determined) before this occurred, his trouble became aggravated; the spleen enlarged still more, the patient emaciated, had irregular fever, and "a long, hard, inflammatory tumor appeared in the region of the right rectus abdominis." This was diagnosed as a myositis and treated with ichthyol, under which treatment it disappeared. Up to this time the diagnosis had been malaria; no blood examination, however, had been made. A few weeks later the attending

* It may here be stated that various affections described, particularly by French writers, under the designation of cutaneous lymphadenia or lymphadenoma, and including manifestly diverse affections, are not considered in this article and, at least for the most part, have nothing to do with true leukæmia.

physician noticed that "there appeared on the skin small nodules, irregularly distributed over the body." They seem to have been observed first on the chest and arms, then on other parts of the body. (See protocol of autopsy below.) Details about their clinical appearance, and how rapidly they grew I was unable to obtain. Their appearance at once led to a diagnosis of multiple sarcoma of the skin. It was subsequently decided to send the patient to New York to a noted specialist for treatment with streptococcus serum, but he was sent back as a case no longer suitable for this treatment. The patient was then referred to me. I suggested a blood examination as imperative, and a blood slide was brought to the laboratory. The picture which it showed agreed well with the clinical history. The enormous increase and the special characters of the white blood corpuscles were at once evident. This unstained specimen showed, besides polynuclear cells, many large mononuclear cells of the type of myelocytes, and a marked increase of eosinophilic cells. A second specimen for staining was requested, but was never obtained, and no opportunity was afforded for making an accurate blood count.

Nothing more of the case was heard until a few weeks later, when I was asked to make the post-mortem examination. Unfortunately the report of this must be nearly as meagre as the preceding history, as the autopsy had to be made in the parlor of the house, without any accommodations, and the bad local custom of injecting a foul-smelling liquid into the abdomen had been promptly followed. Since I was allowed to open only the abdominal cavity, the heart and lungs had to be extirpated from below. The removal of any organ was at first prohibited, but finally I obtained permission to take the spleen, a small piece of the liver, and two of the cutaneous nodules from the left thigh.

Protocol of autopsy (abstracted).—Body of a man apparently between 35 and 40 years old; about 160 cm. tall; extremely emaciated, no panniculus adiposus; muscles atrophied. No rigor mortis. Skin pale. Face shows extreme emaciation; cheek bones very prominent, lips pale and delicate. Scattered irregularly over the body as far up as the neck, numerous on arms and chest, but most marked on the thighs, are small, round, pale, hard nodules, varying from one to two centimetres in diameter, some elevated, all freely movable over the subcutaneous tissue. On incision they show a yellowish-white surface. Chest small, abdomen distended.

On opening the abdominal cavity a large amount of embalming fluid escapes. Small and large intestines distended; serosa pale, opaque, with injected vessels in a few places. The large omentum is very thin and

has everywhere formed thick, firm, fibrous adhesions* with the intestine and rest of the peritoneum.

Quite evident at once is the enormous spleen, which fills the entire left side of the abdomen, extending 8 to 10 cm. below the umbilicus and to the middle line. It is bound by very strong adhesions to the liver and diaphragm, pushing the heart upwards. On being removed, it proves to be 30 cm. long, 17 cm. broad, 6 cm. thick; its weight is 3.0 kg. It is of a dark bluish color and dense consistence. The capsule is very much thickened. On incision it shows a dark red surface; in several places are yellowish spots, the size of a pin's head; at the periphery are a few yellowish, wedge-shaped areas, the bases of which are about one centimetre broad. With the tissues around the spleen an accessory spleen of the shape, size, and color of a plum is removed.

The left kidney, with a fairly extensive fat capsule, is larger than normal and firm; capsule adherent; surface smooth, white, with bluish-red and yellow spots. Cortex broad, pale, dotted with spots of the same character. Pyramids reddened. Right kidney: same condition.

Liver larger than normal; firm; of pale yellow-brownish surface; on section pale yellow; acini distinct.

Heart the size of the man's fist; right side moderately covered with fat: walls normal in thickness, with dark brownish areas; valves normal.

Lungs: in upper portion of a dark red color; the lower portion presents a grayish appearance: emphysematous; the tissue has a crepitant feel, but is soft; on section a large amount of a clear, frothy liquid escapes.

Anatomical diagnosis: Leukæmia; great enlargement of spleen; old splenic infarcts; chronic parenchymatous and hæmorrhagic nephritis; fatty liver; chronic adhesive peritonitis; pulmonary emphysema and œdema; brown atrophy of the heart; circumscribed cutaneous nodules.

The two cutaneous nodules removed at autopsy were fixed in ten per cent formalin solution, which proved very successful, hardened in alcohol, and embedded in paraffin. For a preliminary study specimens were stained in the common way with hæmatoxylin and eosin, later after Biondi-Heidenhain and with methylene-blue according to Unna.

The epidermis and the upper part of the cutis showed no definite changes. The lower part of the cutis, however, was the seat of a

* The "hard long inflammatory tumor in the region of the rectus abdominis," diagnosed as myositis, was undoubtedly one of these strings of thickened peritoneum. I was told that shortly before death several of these masses could be felt and were then regarded as sarcomatous metastases.

dense and diffuse so-called small-celled infiltration. This infiltration extended upward, penetrating the fibres of the cutis, either replacing them entirely or separating them into bundles. Only a very fine reticulum of connective-tissue fibrils remained, which contained greater or smaller groups of cells. In this way some parts of the superficial fat layers were entirely replaced by this infiltration, while others were left relatively undisturbed. Connective-tissue sclerosis, newly-formed blood-vessels, giant cells and necrotic areas or other regressive metamorphoses of cells were absent. The glands were embedded in the cellular masses; they did not show a direct relation to the process. In the further examination of a greater number of sections, especially of one of the nodules, which apparently was more recent, several stages of the process could be demonstrated.

The earliest changes were represented by small, circumscribed focal accumulations of cells situated in the lower part of the cutis. These foci were distinct even to the naked eye, in the microscopic sections, as whitish, tubercle-like formations. As the process progressed, these small nodules became confluent and the appearance then was that of a diffuse infiltration. This was particularly well seen in specimens stained after Biondi-Heidenhain.

In the hæmatoxylin-eosin preparations the largest number of cells possessed irregular nuclei, lobulated and very rich in chromatin, having all the appearances of leucocytes. Only a small number were characterized by fainter, vesicular nuclei, with distinct nucleoli. These latter were regarded as connective-tissue nuclei.

A more careful study of the cells was made in the sections stained after Biondi-Heidenhain and with Unna's polychrome methylene-blue. The largest part of the infiltration was made up of cells presenting the characters of lymphocytes. Of these two kinds could be distinguished: (1) small cells with narrow, faintly staining cytoplasm and deeply staining nucleus, and (2) larger cells with feebly staining cytoplasm and a large, sometimes indented, somewhat irregularly staining nucleus. Besides these two varieties of cells (3) polynuclear leucocytes were common as well as (4) cells with bright red coarse granulations (α-granulation, eosinophilic cells). The large number of

eosinophilic cells deserves especial attention in the light of Nékám's statement that they are found only in recent nodules. In this patient the cutaneous lesion did not appear until a few months before death, and the two nodules taken from the thigh for examination were apparently of recent formation. If red blood corpuscles escape in any large amount, they must certainly be removed very rapidly, for they were not at all conspicuous in my specimens. In only a few sections did I occasionally see a large cell with large eccentric nucleus which answered to the characters of plasma cells. But these cells were compressed and their view obstructed by the great number of surrounding leucocytes. Plasma cells, if present at all, were certainly in such small number as to form no characteristic part of the cellular accumulations. Mitoses were not observed, and while it is desirable to examine material from a larger number of cases before passing a final judgment upon their occurrence in leukæmic cutaneous nodules, their absence in both Nékám's and my cases indicates that they are not constant or essential features of these nodules.

In view of Unna's statements concerning the nature of leukæmic nodules in the skin, the absence of plasma cells, at least in noticeable quantity, is of interest. It may be recalled that Unna, who first distinguished these cells, believes that they are derived from connective-tissue cells, but the subsequent studies of Marschalkó, Justi, and Councilman* have demonstrated that they come from lymphocytes. Plasma cells are found especially in infectious granulomata, in granulation tissue, and in many of the so-called inflammatory round-celled and lymphoid-celled infiltrations of organs. We possess no information at present concerning the occurrence of plasma cells in leukæmic nodules in the liver and other parenchymatous organs. Their absence from these nodules in the skin, noted in Nékám's and my cases, is noteworthy, in view of the predominance of lymphocytes in these lesions, and, taken in connection with the absence of mitoses, speaks against the occurrence of active cellular growth and multiplication in any considerable degree within these nodules. As already

* *Journal of Experimental Medicine*, 1898, iii, 393. Consult also for other references and a full consideration of these cells.

indicated, the connective tissue is the seat of atrophy at the site of the cellular accumulations, and does not participate by proliferation of the fixed cells in the formation of the nodules. It furnishes a kind of reticulated stroma for the cells.

The histological examination of the leukæmic cutaneous nodules in my case, therefore, supports the view of those who regard secondary leukæmic nodules as essentially composed of cells derived from the blood. I am not inclined to lay great stress upon the failure to find karyokinetic figures in the cells, as indicative of a distinction between these nodules in the skin and those so often found in leukæmia within parenchymatous organs, but upon this point further observations are needed. Why the skin should be such an uncommon seat of secondary leukæmic nodules, thus contrasting with the liver and kidneys, it would be at present useless to speculate.

The propriety of designating these nodules as lymphomata cannot be profitably discussed until there is greater agreement of opinion, than at present, concerning the definition of what shall be called "lymphoma." As this term etymologically suggests an actual new growth or tumor, its application to such nodules as those described in this paper seems to me of at least questionable propriety, as it is clear that these nodules are not genuine neoplasms.

I desire to express sincere thanks to Professor Dunham of the University and Bellevue Hospital Medical College for kind assistance in the preparation of this article and to Professor Welch of the Johns Hopkins University for helpful suggestions.

A CASE OF ADDISON'S DISEASE WITH SIMPLE ATROPHY OF THE ADRENALS.*

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PLATE XXVIII.

The study of simple atrophy of the adrenal bodies in Addison's disease may prove of considerable importance in explaining more thoroughly the process underlying this group of symptoms. The lesions in simple atrophy of the adrenals afford a much better opportunity of investigating the gradual death of the parenchyma than the gross destructive changes in tuberculosis of the glands, most commonly associated with the disease. The histological examination of the comparatively early phases of the atrophic lesions in the present case possesses considerable interest, for the subject has received but little investigation. Very few cases of simple atrophy of the adrenals in Addison's disease are recorded, and of these hardly a single one has been given thorough histological study.

I am indebted for the following clinical notes to Dr. D. H. McAlpin, Jr., Visiting Physician to the City Hospital of New York.

F. N., male; aged 42; admitted to City Hospital August 10, 1897, died February 17, 1898.

Family history: Negative.

Previous history: During childhood the patient had measles. At 20 years of age he had an attack of rheumatism, after which he was well until his 28th year, when he had some affection of the skin. Patient denied alcoholic excess and syphilis.

Fourteen years ago, he says, he began to "turn yellow," but he was well for seven years after the first appearance of the discoloration, when he

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had another attack of rheumatism in the legs, from which he soon recovered. In 1895 he had a similar attack of "rheumatic" pain in the legs, accompanied by acute bronchitis, loss of flesh, and profound weakness, but he recovered and was apparently well until June, 1897. Six weeks before admission, he had an attack of weakness, diarrhœa and polyuria, without headache or pain in the abdomen. The feet became swollen and he had pains in the legs and ankles.

Status on admission: The entire body is of a yellowish-brown color, deeper in tint where protected from the sun, and especially dark over areas pressed upon by the clothing, waistband, collar, suspenders, etc. Ocular conjunctivæ pearly white. Mucous membrane of tongue and mouth pale and free from pigment. The patient's mental condition is dull. He says he has a fair appetite, sleeps well, but has pains in the knees and ankles. The bowels move three to four times daily. He complains also of epigastric pain and slight cough.

Physical examination of lungs revealed high-pitched and rather prolonged respiration at inferior angle of left scapula. Blood examination, September 6, by Dr. Reilly; hæmoglobin, 20 per cent; reds, 1,490,850; whites, hypoleucocytosis. From the stained specimens, I made the following observations: Differential count of 500 leucocytes gave: adult cells, 55 per cent; young cells, 41 per cent; eosinophiles, 4 per cent; myelocytes absent. There are a few microcytes and poikilocytes but the blood does not indicate severe anæmia.

September 11. Patient complained of severe darting pains from the neck to the occipital region and of pain in the left hypochondrium. For the past 18 days he has had frequent stools. At this time examination showed impaired resonance over both infraclavicular regions with fine moist rales, heard especially posteriorly.

September 13. On ophthalmoscopic examination, retinæ and optic disks were extremely pale, but otherwise normal. The following week the patient seemed much improved. The cough became less frequent and severe, the stools less numerous and he appeared to be brighter mentally. On September 22, diarrhœa recurred with frequent bilious vomiting, watery stools, cough, pain in the abdomen and legs. This condition persisted except at intervals of a few days during which the patient would feel fairly comfortable. On October 26, ptosis of the right eyelid developed, but disappeared after a few days.

On physical examination, November 5, bronchial breathing, coarse mucous rales, friction, etc., were heard over the right infraclavicular region, but there was no evidence of cavity formation.

The patient remained in this condition until January 15, 1898, after which date he began to grow much weaker. The diarrhœa and vomiting continued with severe abdominal pain and at times delirium. He died February 17, 1898. The temperature was irregular and atypical and at no time above 101° F. The urine was examined weekly. The specific gravity varied from 1018 to 1022. Neither albumin nor sugar was found.

The *autopsy* was performed a few hours after death. I have prepared the following notes from Dr. McAlpin's protocol.

Body extremely emaciated; eyes and cheeks sunken; panniculus adiposus absent. Skin of entire body of a brownish mulatto color, pigmentation being especially pronounced over upper anterior part of thorax, scrotum, glans penis, dorsum of hands and feet, and either side of spinal column, especially in lumbar and sacral regions. No pigmentation of buccal mucous membrane or sides of the tongue. Conjunctivæ generally white, but tinged with yellow where exposed to the air. Peritoneum smooth and glistening. Stomach and large intestine distended with gas. Lower edge of right kidney $1\frac{1}{2}$ inches below last rib; left kidney same, slightly movable. Vermiform appendix small, its lumen patent and filled with impacted hard fæcal matter. Bladder distended. Edge of liver above free border of ribs; gall-bladder distended.

Right lung: Oedematous; a few old adhesions over middle lobe; cut section of lower lobe yellowish pink, with small, elevated, pale, dry, granular areas exuding a creamy fluid on pressure; upper lobe grayish yellow, with moderate anthracosis, and a few apical caseous nodules from 1 to 6 cm. in diameter.

Left lung: Oedematous; old adhesions over upper lobe; tubercular nodules at apex and a few in lower lobe, surrounded by firm, pinkish granulations. Bronchial glands enlarged and the right calcified.

Heart: About 20 cc. clear serum in pericardial sac. Heart of average size; epicardial fat absent; right ventricle collapsed; right auricle distended with ante-mortem clot; cavity of left ventricle dilated; walls thin, pale brown; endocardium normal; aortic and mitral segments practically normal; no myocarditis; right ventricle normal; tricuspid and pulmonary valves normal, save some yellowish discoloration of segments; also some atheroma of coronary arteries.

Liver: Small, reddish-brown, firm; lobules distinct, with dark centres; vascular walls thickened. Gall-bladder distended with two ounces of dark greenish, thick bile, containing many small, pultaceous masses; gall-ducts patent; mucous membrane normal.

Stomach: Small, mucous membrane normal.

Pancreas: Pale, very firm, evidently the seat of interstitial hyperplasia.

Small intestine: Extremely pale, distended with gas; mucosa pale, covered with mucus; no increase of adenoid tissue.

Mesenteric glands small, firm.

Spleen: Somewhat enlarged, firm, reddish-brown.

Bladder distended with eight ounces of dark urine, mucosa normal.

Kidneys: Enlarged; finely granular surface, in places cystic; cortex thickened; pale yellowish, striæ obscure, small, grayish, tubercle-like bodies on surface.

Adrenals: The left measures 2 in. in length by $\frac{3}{4}$ in. in width and less than $\frac{1}{4}$ in. in thickness; the right has about the same dimensions; their general appearance normal, except somewhat atrophic; surface smooth and pale; consistence normal; no evidence of tubercular disease.

Cœliac sympathetics easily removed and apparently normal.

Brain: Small, fresh hæmorrhages on under surface of dura, especially on right side. Pia mater very œdematous, opaque and thickened; in region of both Sylvian fossæ, dusky pigmentation, seen also over pons. Brain small, symmetrical and very pale; sulci deep, convolutions thin.

Spinal cord: Normal.

Anatomical diagnosis: Chronic miliary tuberculosis of lungs; tuberculosis of bronchial glands; passive congestion of liver; chronic interstitial pancreatitis; chronic catarrhal enteritis; chronic interstitial nephritis, and simple atrophy of adrenal bodies.

Microscopic examination: Unfortunately as the central nervous system was not preserved and the dissection of the sympathetic was incomplete, the microscopic examination is restricted to the only available material, the adrenal bodies and the semilunar ganglia.

Pieces from both *adrenals* were hardened in formaldehyde solution (4 per cent), in absolute alcohol, and in Müller's fluid. The entire gland of either side was cut and stained. The sections were variously stained in Delafield's hæmatoxylin and eosin-blue, lithium carmine, Van Gieson's mixture of picro-acid-fuchsin, by the Heidenhain-Biondi method, etc. The microscopical findings being similar in both adrenals, one description will suffice. On casual observation the appearance of many sections is that of normal tissue, but on closer examination and by careful comparison with a series of sections taken from normal adrenals distinct morbid changes can be demonstrated. The periglandular fat is decreased in amount and the seat of serous atrophy. The remains of the adipose tissue are seen as a richly cellular mass, in which appear fully formed fat

cells. The intercellular substance is a homogeneous finely granular material, in places fibrillated, which encloses small, round, deeply stained nuclei. The fibrous capsule, with the septa, does not appear thickened and is free from evidences of inflammation and of tuberculosis.

The zona glomerulosa is present in some part throughout all the sections, the individual glomeruli varying greatly in size and appearance. In places they are represented by only one or two small cells, with round, deeply stained nuclei, and at times they appear to lie in the outer capsule. In places, however, this zone appears two or three times the usual thickness and is composed of four or five layers of glomerular acini, and again in other areas it is entirely absent, the cells of the zona fasciculata being in contact with the fibrous capsule.

The part of the gland represented by the zona fasciculata shows many interesting as well as uncommon conditions. The most striking change consist in the irregularity of the columns in this portion. The cells, instead of being arranged in definite columns, running more or less vertically from the zona glomerulosa to the medulla, compose branching masses and columns, having an irregular course and being intimately mingled with cells from the zona reticularis. The cells vary greatly in size and appearance, their nuclei being small, round, deeply stained dots. The cytoplasm is finely granular and stains a delicate pink with a watery solution of eosin-blue. The walls of the individual cells cannot be distinguished, so that the general appearance is that of finely granular, branching masses, in which lie small, round nuclei. The small cells, which are arranged in tubules in this region, have protoplasm resembling in every way that of their enlarged associates. The fatty condition of the cells, normally present in this region, is absent or noticeably diminished and in only a few sections was I able to demonstrate the large, clear fatty cells, so common in the normal organ.

Especially in specimens hardened in Müller's fluid, are seen in the fascicular zone circumscribed areas composed of large, pale acini, containing a peculiar colloid material of crescentic and other shapes. These colloid masses, when stained by eosin, contrast by their brilliant red color with the pale pink cytoplasm. With picro-acid-fuchsin they are colored a brownish-purple. They have sharp borders and are striking objects in the sections. Auld describes cylindrical bodies with highly refractive index between, or more often in, the medullary cells of adrenals hardened with chromic salts. Colloid masses similar to those described, however, I have never seen in any other sections from a series of at least fifty adrenals studied during the past year.

Adjacent to the swollen areas of the zona fasciculata are seen tubules made up of small cells which have been pushed aside and evidently compressed. Such a condition is commonly seen in the normal gland just at the side of a small area undergoing fatty metamorphosis.

The most prominent departure from the typical structure, is seen in the region immediately adjacent to and including the upper medullary portion or in the zona reticularis (Plate XXVIII, Fig. 2). In specimens hardened in alcohol, there is an evident increase in the normal content of pigment, of a bright, brownish-yellow hue, both in the cell bodies and free in the intercellular substance. So great is the accumulation of pigment granules that sections stained with Delafield's hæmatoxylin and eosin-blue do not reveal the nuclei in this region. Potassium ferrocyanide and dilute hydrochloric acid fail to give evidence of iron in the pigment granules. The cells of the zona reticularis, instead of appearing in small anastomosing columns, remain more or less distinct in clusters of two or three. This appearance is to be interpreted as evidence of simple atrophy.

The intercellular spaces are large and prominent and appear as finely granular areas, in which delicate threads of the normal reticulum are distinct.

The polymorphism of the cells of the zona reticularis is very noticeable. They vary from one-third to twenty times the size of the typical cell in this situation. Many large homogeneous cells of giant size are seen (Plate XXVIII, Fig. 1). Each of these possesses a single large, deeply stained nucleus, usually about the centre of the cell. Many of these nuclei have a diameter ten times that of those in adjacent cells. The cytoplasm appears almost homogeneous, stains with eosin a delicate pink and, without definite limits, shades off insensibly into the surrounding intercellular substance. These I take to be hypertrophic cells undergoing necrobiosis. Many of the smaller cells in this zone also show evidences of degeneration and necrosis. Many are polychromatophilic with indistinct fragmented nuclei; others appear swollen, granular and devoid of nuclei, while in places the original cell is represented by nothing but a small mass of pigment.

In this region, as already mentioned, the intercellular substance and fibrous reticulum, are especially prominent, less apparently from actual new formation, than on account of the atrophy and disappearance of the columns of cells. Sections treated with picro-acid-fuchsin, fail to show any definite increase of the fibrous stroma. The reticulum is throughout rich in round and fusiform, deeply staining nuclei and especially so about the smaller capillaries.

The blood-vessels of the adrenals show in many places a pronounced perivascular infiltration with small round cells. The walls of the larger vessels present in areas peculiar nodular thickenings, not unlike those first described by Kussmaul, as periarteritis nodosa. There is a moderate nodular fibrous thickening of the intima of the vessels, but not so marked as to encroach materially upon the lumen.

The medullæ of both adrenals show but little microscopical evidence of disease. Here and there are seen, embedded in the medulla, portions of the cortex, a finding not uncommonly met in the normal organ. This portion of the gland is relatively small in amount, but present in all the sections near the hilum. As the edge of the gland is reached, it becomes narrower and ceases entirely about 1 cm. from the free edge. At the junction of the medulla with the cortex, I was able by the Nissl method to demonstrate here and there ganglion cells staining deeply and showing the same picture as that seen in cells of the semilunar ganglia.

Sympathetic ganglia: The right and left semilunar ganglia were hardened in absolute alcohol. Sections were stained by the Nissl stain, Van Gieson's method and hæmatoxylin and eosin. With simple stains the ganglion cells appeared numerous, without evidence of atrophy or degeneration. With the Nissl stain, the cells appeared large and fully formed, staining deeply and without evidence of chromolysis. At the borders of many of them were seen dark brownish metaplasm granules, usually appearing as a little irregular cluster on one side of the cell. The Nissl bodies were fully formed, standing out distinctly in the cytoplasm. A few of the ganglion cells contained two well-formed and distinct nuclei. This condition has been observed a few times before in different conditions, but is not believed to have any pathological significance. Nor to the increase of pigment can especial significance be attached. Otherwise no lesion is apparent. There was no small-celled infiltration, necrosis, caseation or any condition even suggestive of simple or tubercular inflammation in the cœliac ganglia.

The following facts in this case of Addison's disease seem to me worthy of note: the appearance of the cutaneous pigmentation fourteen years before the onset of the profound constitutional symptoms; the occurrence of severe rheumatoid pains in the extremities, so commonly observed among the early manifestations of the disease; the profound weakness with uncontrollable diarrhœa, epigastric pains and bilious vomiting; the prolonged duration of life (six months)

after the onset of the severe diarrhœa and vomiting; the irregular and atypical temperature; the absence of albumin or sugar from the urine; the characteristic color and distribution of the pigment; the rather severe anæmia with hypoleucocytosis.

The condition of the adrenal bodies found at autopsy was certainly surprising, the only gross abnormality being a simple atrophy of quite moderate degree. The microscopical evidences of this condition may be briefly summarized as follows: diminution in size and number of the glomeruli of the zona glomerulosa; diminution in length with great irregularity in the course and arrangement of the columns, as well as diminution of their fatty contents; colloid degeneration of circumscribed areas in the zona fasciculata; marked atrophy with increased pigmentation of the cells in the zona reticularis and replacement of some of the cells by clumps of pigment; general diminution in size of the medulla associated with the presence of displaced cortical structures; the presence of many mononuclear cells of gigantic size, of cells with fragmented nuclei and cells devoid of nuclei; thickening of the vessel walls with perivascular infiltration, and absence of chronic interstitial or tubercular inflammation.

These conditions warrant the diagnosis of *simple atrophy*, associated, perhaps, with attempts at compensatory cellular hypertrophy.

Assuming this condition, without lesion of the abdominal sympathetics, to be the essential cause of the clinical symptoms in this case, it may be well to call to mind other conditions concerned with the pathogenesis of this disease.

Addison, in his original memoir, considered that any lesion of the adrenal bodies, which interfered sufficiently with their function, could give rise to the disease.

Guy and Fowler, however, found that malignant lymphadenoma destroying the cœliac ganglia, without involvement of the adrenals, was capable of causing the classical symptoms. Rolleston, in the Goulstonian lectures for 1895, gives the following morbid conditions in the adrenals as possible etiological factors: Caseating tuberculosis; simple atrophy; chronic interstitial inflammation; malignant disease; occlusion of the adrenal vein; hæmorrhagic states. To these he adds destructive lesions of the semilunar ganglia.

From this classification, we are to believe that next to tubercular disease of the adrenal bodies, simple atrophy is the most common cause. Examination of the records shows reports of at least 16 cases of atrophy of the adrenals associated with symptoms of Addison's disease.

The cases reported by Osler,* Monti,† and Hadden,‡ respectively, should be classified as degenerative atrophies associated with chronic interstitial inflammation. There thus remain for consideration only 13 cases. Brief citations from the various records may not be out of place here:

Case I. (J. K. Spender, *Brit. Med. Journ.*, 1858.) Female, aged 53. Time of the first appearance of the pigmentation unknown. Attacks of profound prostration, epigastric pain, bilious vomiting and diarrhœa. Skin of entire body of a yellowish-brown color. At autopsy entire absence (?) of both adrenals.

Case II. (J. W. Legge, *St. Bartholomew's Hosp. Rep.*, 1874, X.) Female, aged 37. 18 months before admission noticed dark brownish streak on lip; 6 months ago, face became pigmented and there appeared weakness, shortness of breath and frequent attacks of bilious vomiting. Yellowish-brown color over entire body, especially over face, neck and dorsum of hand. Small patches of pigment on buccal mucous membrane. Symptoms became exaggerated and patient died one month after admission. At autopsy no evidence of tuberculosis in the body. The right adrenal absent but replaced by a mass of fat supplied by the adrenal artery and vein. The left adrenal thin, fibrous, without trace of the normal cortex. Microscopic examination revealed fully formed connective tissue, fat cells but no remnants of the gland. No coarse disease of nerves or ganglia.

Case III. (Goodhart, *Trans. Lond. Path. Soc.*, 1882.) Coal miner, aged 20. Admitted October 7, 1881, on account of increased weakness. Skin had begun to darken two years before. Pigmentation appeared first upon glans penis, then on face, arms, legs, etc. One year before admission attacks of severe epigastric pain with nausea. Skin dry, of a marked brown color, especially on sides of body, buccal mucosa, nipples, scrotum and glans penis. The diarrhœa, vomiting and weakness in-

* *Internat. Med. Mag.*, 1896-7, v. 3.

† *Arch. f. Kinderh.*, 1884-5, vi, 319.

‡ *Trans. Lond. Path. Soc.*, 1885, xxxvi, 436.

creased and the patient died in coma. Aside from the hyperplasia of the adenoid tissue of the gastro-intestinal tract, the principal post-mortem findings were in the adrenals. In the place of each of these bodies was a thin layer of a reddish-brown substance, still retaining the triangular outline of the gland and connected with the large adrenal vein. The glands were so thin, that, when placed between the fingers, they could hardly be appreciated. Microscopically the tissue showed simple atrophy without evidence of tubercular disease or implication of the cœliac ganglia.

Case IV. (Goodhart, *Trans. Lond. Path. Soc.*, 1882.) Draper, aged 44. For twelve years has had rheumatic pains in the knees. Eight years before skin became pigmented. Acute melancholia, necessitating confinement in a sanatorium, appeared for some months, but from this there was entire recovery. For a month before death, emaciation, weakness, nausea, vomiting and epigastric pains were manifested. At autopsy all organs healthy, except the adrenals, which were so wasted as to be scarcely visible. No evidence of suppuration or of caseous material. Apparently there was simple atrophy until each gland was reduced to the size of a split pea.

Case V. (Davy, *Trans. Lond. Path. Soc.*, 1882.) Machinist, aged 25. 3 years before entrance to hospital noticed pigmentation of nose, followed shortly by pigmentation under eyes. Gradually entire body became yellowish-brown. At times profound prostration. Death in coma following alcoholic intoxication. Entire surface of body uniformly pigmented, especially over genitals, flexor surfaces of joints and axillæ. Conjunctivæ pearly white, pupils minutely contracted. Left adrenal very much smaller than usual but of normal appearance except in one place near the middle. Right adrenal presented even less recognizable structure. Semilunar ganglia not found on account of fat.

Case VI. (R. G. Hebb, *Lancet*, 1883, I.) Female, aged 48. Pigmentation of skin noticed four weeks before, preceded by emaciation, attacks of headache, vertigo, nausea, vomiting and constipation. General pigmentation, especially over neck, elbows, areola of nipples and buccal mucosa. At autopsy no evidence of tuberculosis. Adrenals weighed 28 and 19 grains respectively, were of normal shape, of reddish color and soft, medulla apparently absent. No lesion of sympathetic ganglia observed.

Case VII. (Sidney Coupland, *Trans. Lond. Path. Soc.*, 1885.) Stone-sawyer, aged 34, admitted June, 1881. Present ailment began two years ago with loss of appetite, vomiting, weakness and pigmentation of skin, which became deepest on face, dorsum of hands, neck and buccal

mucous membrane. Patient had many attacks as described and was under observation two and a half years. In January, 1884, following over-indulgence in alcohol, he succumbed, but was conscious until the last few hours. Mesenteric glands and Peyer's patches enlarged. No trace of right adrenal—possibly replaced by a small mass of fat. Left adrenal one-third normal size, of usual shape, very thin and almost translucent. Semilunar ganglia normal.

Case VIII. (Thomas Barlow, *Trans. Lond. Path. Soc.*, 1885.) Female, aged 42, admitted July, 1883. Pigmentation of skin began one year before; pregnant for last nine months; attacks of weakness and vomiting. On July 1, birth of a still-born child, followed by great prostration and death, in a typhoid state, a short time afterwards. At autopsy, pigmentation of entire body, deepest over areola of nipples, groins, dorsum of feet, under side of knees, etc. At first it was believed that the adrenal bodies had vanished without a trace, but careful dissection indicated the fibrous investment of the bodies, still preserving their cocked-hat shape and containing the merest trace of pale glandular tissue in each, which on microscopical examination gave evidence of the columnar arrangement of the cortex.

Case IX. (J. W. Legge, *Lancet*, 1885.) Female, aged 29, admitted May, 1884. Six months before had an attack of vertigo with vomiting. One month later noticed pigmentation of the skin, of a tawny brown color, deeper over right eye, xyphoid cartilage and axillæ. Clinical course characterized by fever, vomiting, progressive asthenia, deepening of the pigmentation and death. Autopsy: Lungs normal; right adrenal represented by a small shred of tissue about one-half inch long and one-quarter inch broad, left black, of natural outline, but completely wasted and thin as paper; solar plexus to naked eye normal.

Case X. (Senator, cited from Lewin, *Charité-Annalen*, 1885, p. 648.) Female, aged 53. For past six years attacks of vomiting; six weeks ago black masses of blood vomited, at same time high fever and epigastric pains. One month ago, small dark spot appeared on the gums. Soon entire body became bronzed, the pigmentation being especially marked upon the face. Many dark brown spots on face, arms and groins; skin, especially of fingers, rough and scaly; hair completely gray; iris yellowish-brown, sclera somewhat gray. Albuminuria with hyaline casts and pus corpuscles. Pulse 152. Adrenals markedly atrophic, the cortex being represented by scarcely more than two separate leaflets.

Case XI. (P. Guttmann, cited from Roloff, Ziegler's *Beiträge*, ix, p. 344.) Male, aged 20. One year before noticed pigmentation of the

skin, followed by increasing weakness, vomiting, epigastric pains, sopor and death. Autopsy: pronounced brownish pigmentation, most marked upon face, mucous membrane of mouth and lips, genitals, nates and bend of knees. Induration of pulmonary apices. Both adrenals reduced to mere traces of cortical substance. Cervical (? cœliac) ganglia normal.

Case XII. (Roloff, Ziegler's *Beiträge*, ix, p. 329.) Male, aged 22. Pigmentation, which increased in intensity, began 13 years previously, after an attack of acute pleurisy. Patient was comparatively well until five days before death. Constitutional symptoms began with loss of appetite and weakness. The day before his death, for the first time had vomiting and epigastric pains. He soon became delirious and died. Autopsy: acute hyperplasia of the gastro-intestinal lymph nodes; chronic pulmonary tuberculosis; right adrenal, 20x12x3 mm., markedly atrophic, left a little larger, but not more than $\frac{1}{4}$ to $\frac{1}{5}$ usual size, without evidence of tuberculosis in either gland, either macroscopically or microscopically. Microscopical examination revealed total absence of fat in the cortical cells. The principal portion of the gland remaining is the medulla. Here and there are small islets of tissue resembling the zona glomerulosa and zona reticularis. Microscopically semilunar ganglia normal.

Case XIII. (Bramwell, *Brit. Med. Jour.*, 1897.) Grocer, aged 37. Typical case of Addison's disease, characterized by profound and causeless asthenia, pigmentation of skin and buccal mucous membrane, pain in neck, occasional vomiting, slight emaciation. Death from influenza. Autopsy: lungs normal; complete absence of adrenals, their place being taken by masses of fat, which on the left side presented appearances suggestive of the remains of the degenerated adrenals. Microscopical examination revealed only a mass of fat cells and blood-vessels without trace of glandular structure.

It is seen that of the 14 cases reported (including my own), tuberculosis was present in the body only three times, being absent in all instances from the adrenals themselves. In none of the 13 cases from the records was the lesion of the glands relatively so slight as in the present case. The adrenals in case XII were apparently the largest of the series, and even the largest was less than 3 cm. in its greatest diameter. In my case they were nearly of normal size.

In two of the cases (I and XIII) the glands were apparently entirely absent, suggesting complete aplasia. Zander and others have noted

such a condition, but always, so far as I am aware, it was associated with defective embryological developments in other portions of the body, and it can hardly be supposed that the patients could have survived so long with actual aplasia of the adrenal glands. Case XII is at least suggestive of hypoplasia. Here the pigmentation of the skin began when the individual was nine years of age and continued for thirteen years, his health remaining fairly good until five days before death. The pathogenesis of this form of simple atrophy of the adrenal bodies is entirely unknown.

In attempting an explanation of this condition, the general causes of simple atrophy may be referred to: (1) pressure; (2) disuse; (3) senility; (4) neuropathic influences; (5) impaired nutrition. The first three need not be discussed. The possibility of defective inheritance may be suggested, but of its participation in the etiology of the disease we have no conclusive evidence.

In regard to neuropathic influences, there is evidence that lesions of the sympathetic may produce indirectly atrophy of the adrenal bodies, and as a result the symptom-complex of Addison's disease. Whether or not the ganglion cells have a trophic influence upon the cellular parenchyma of the adrenals, analogous to that of the anterior motor cells of the spinal cord upon voluntary muscular fibres, is not positively known, but recent physiological experiments fail to strengthen this supposition. von Kahlden and others have shown that a lesion involving the splanchnic nerves without direct lesion of the semilunar ganglia, may produce these clinical symptoms, and Biedl has shown that the secretion of epinephrin is under the influence of these nerves. In our case the splanchnic nerves were not carefully dissected out and examined, but it is certain that no gross or histological changes were present in the cœliac ganglia.

It has been suggested that certain cells in the spinal cord may exercise a trophic influence upon the adrenal parenchyma, but there is only negative evidence to support this hypothesis. The brain and cord of the present case were not obtained for microscopic examination.

Defective nutrition cannot be excluded as a possible causative factor in this case, but no such changes were observed in the adrenal vessels as would indicate serious disturbances in the local circulation.

It must be admitted, therefore, that at present we are as much at a loss to understand the pathogenesis of this condition as was Spender in 1858.

The work of Oliver and Schaefer,* and more recently that of Abel,† has shed light upon the function of the adrenals. The blood-pressure-raising constituent, designated by Abel "epinephrin," has been shown to be derived from the medulla, but the exact rôle that it plays in normal metabolism or in the production of the pigmentation and other phenomena of Addison's disease has not been demonstrated. The medulla in the glands of the case just reported, though as a whole somewhat diminished, is nevertheless present in comparatively fair amount.

It is difficult to believe that the symptom-complex of Addison's disease can be explained by such quantitatively insignificant histological changes as were present in this case. It is well known that certain glandular organs may undergo perversion of function and still present little or no histological evidence of disease. There is evidence that some cases of diabetes mellitus are referable to disordered function of the pancreas without manifest histological lesions of this organ. Recent investigations have demonstrated the vital importance of the adrenal glands and their endowment with a so-called internal secretion. If it were possible to estimate clinically the amount of epinephrin secreted, as we estimate the amount of urea in the urine, we might then be in a better position to explain such obscure case of Addison's disease as the one reported in this paper. Although Addison's disease is undoubtedly due to some interference with the function of the adrenal bodies, it seems probable that, in this case at least, we have to deal with a perversion rather than with a total lack of function.

DESCRIPTION OF PLATE XXVIII.

Fig. 1. From section of adrenal. *A*, large cell with vacuoles; *B*, cells of the zona reticularis; *C*, perivascular infiltration. Zeiss ocular 2, objective D.

Fig. 2. Section of adrenal. *A*, portion of normal zona fasciculata; *B*, simple atrophic cells of the zona reticularis; *C*, atrophic and hypertrophic

* Oliver and Schaefer, *Journal of Physiology*, xvi and xviii.

† Abel, *Bull. of the Johns Hopkins Hospital*, 1897, p. 153, and *Zeitschr. f. physiol. Chemie*, xxviii.

cells in contiguity; *D*, pigmented protoplasmic remains of cell bodies; *E*, connective tissue reticulum prominent on account of degeneration and disappearance of the parenchyma; *F*, normal cells in zona reticularis.

The red masses seen in some of the cells correspond in reaction to the colloid masses described as occurring in the zona fasciculata.

Technique. Hardened in Müller's fluid, stained in Delafield's haematoxylin and one per cent aqueous solution of eosin-blue. Zeiss ocular 2, objective D.

FIG. 1

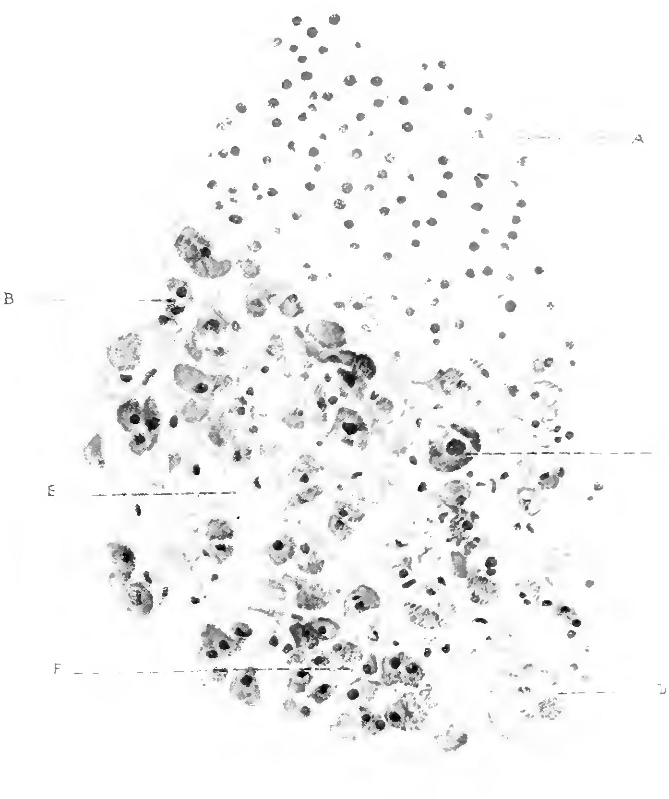

FIG. 2

A PECULIAR FORM OF FIBROSARCOMA OF THE BRAIN.

By ALICE HAMILTON, M. D.

(From the Pathological Laboratory of the Woman's Medical College, Northwestern University, Chicago.)

PLATES XXIX AND XXX.

Fibrosarcoma of the brain, though described with relative frequency by earlier writers, scarcely finds mention in later text-books of pathology, and though individual cases are still recorded in the current literature, they almost invariably come from observers who base their diagnosis chiefly upon the gross appearances, and whose description of the microscopic findings is far from satisfactory. A careful examination of the literature on the subject forces the conviction that the majority of tumors which have been classed as fibrosarcomata were in reality gliomata unusually rich in fibres. Indeed, the tendency of pathologists now is to regard as gliomata all tumors of the nervous system, which cannot be proven to have taken their origin either in the meninges or in the walls of the blood-vessels, in the latter case usually in the endothelium. It is, therefore, with some hesitation that I venture to give the name of fibrosarcoma to a primary growth in the cerebrum, a growth which cannot have originated in the meninges nor in the endothelium of the vessels. It seems, however, impossible to class it otherwise than as a connective-tissue tumor of a very peculiar type, originating probably in the outer coats of the blood-vessels.

The specimen was given me for examination by Dr. L. L. Skelton, of Chicago, to whom I am indebted for the following brief notes of the clinical history and autopsy. The patient was a married woman, twenty years of age. When seen by Dr. Skelton in consultation she was suffering from "terrific" headaches, and gradual impairment of vision. There were slight incoördination, hysteria, psychic changes

and intermittent albuminuria. Ophthalmoscopic examination showed choked disks. Dr. Skelton diagnosed a tumor of the brain, and advised operation. This was refused, and he did not see the patient again until after her death, when he was requested by the family physician to make an autopsy. This was done twenty-four hours after death. Upon removing the skull-cap the right cerebral hemisphere was seen to bulge a little, but the meninges and cortex were apparently unaltered. When, however, a shallow incision was made in the frontal region through the cortex, the latter peeled away, leaving exposed a spherical mass distinctly circumscribed and readily enucleated. This mass occupied the deeper parts of the three frontal convolutions and subjacent white substance projecting into the ventricle, but not involving the basal ganglia. No important changes were found in the thoracic or abdominal viscera; there were no new growths in any organ save the brain.

The specimen, when it came into my hands, had been for eighteen months in a four-per-cent solution of formaldehyde. Unfortunately the brain had not been preserved in toto, but the tumor had been removed with only that part of the tissues which directly surrounded it, so that the exact relation of tumor to brain could not be determined. The mass was about as large as a medium-sized orange and firm in consistence; its color was whitish with gray streaks. The cerebral tissue separated easily from the tumor; in no place was there any sign of infiltration, extension of the growth having evidently taken place by displacement of the normal tissue. Pieces from different parts of the tumor were hardened, some in alcohol, others in potassium-bichromate-chromalum solution according to Weigert's quick method for myelin sheaths, and others in picric acid and ammonium bichromate for Mallory's neuroglia stains. Sections were stained in the above-mentioned ways, and also with hæmatoxylin and eosin, with Van Gieson's picro-acid-fuchsin, and with Weigert's new fuchsin stain for elastic fibres.

The first sections examined were those which had been cut from the edges of the tumor, where the new growth seemed, to the naked eye, to be sharply divided from the uninvaded brain tissue; this im-

pression was confirmed under the microscope. The tumor was circumscribed, although not encapsulated, and there was no sign of a gradual infiltration of surrounding parts. Furthermore, the meninges were found to be uninvolved in the new growth. Often the normal tissue was separated from the tumor mass by a prolongation of the pia mater in between two convolutions, while the membrane itself was normal save for slight round-cell infiltration.

Examined under the low power of the microscope, the tissue of the tumor was seen to be exceedingly rich in cells, closely crowded together, and with very scanty intercellular substance. The cells were not evenly distributed, being often gathered together in clumps, which seemed composed of more or less circular elements, while bands of spindle-shaped cells occasionally ran across the field between the clumps. Spindle cells could also be seen near the blood-vessels, and these cells were possessed of processes, while the circular and oval cells were apparently devoid of processes. The intercellular substance had usually a granular appearance, but between the spindle cells it was delicately fibrillar. Under the high power the cells exhibited a great variety of shapes and sizes. So closely were they packed together in some places that it was impossible to distinguish their individual outlines, the appearance being that of a large mass of protoplasm with nuclei scattered through it. In thinner places, however, the cells stood out clearly, the protoplasm of the largest ones being granular, and taking the stain deeply. Mallory's phosphomolybdate stain brought out the cells most distinctly, and sketches were made of the different types in sections stained after this method (Plate XXIX, Fig. 1). The cell which seemed most widely distributed was the round, or irregularly oval, or angular cell of medium size, with lightly stained protoplasm and a single nucleus, the latter usually circular, staining deeply at the periphery and showing coarse chromatin granules. Apparently naked nuclei, singly or in clumps, occurred with great frequency, and occasionally very small cellular elements were found with a pyriform body and one delicate process. The spindle cells, mentioned above, occurred, not scattered among the other cells, but in slender bands or close to the blood-vessels. They

possessed but one nucleus, and one or more processes at each end. Some of them resembled the so-called brush cells. They lay with their long axes parallel, and their processes formed a fibrillar matrix, which was wanting elsewhere in the tumor. The larger cells were most irregular in outline and in the arrangement of their nuclei, which were sometimes as many as ten in number. The protoplasm was more granular than that of the other cells, and stained deeply; the nuclei were richer in chromatin. These cells were often found in groups, but no part of the tissue was totally devoid of them. They were frequently vacuolated, and this gave rise to appearances like those interpreted by some observers as intracellular parasites. Sometimes these cells would have one delicate process, rarely more, but the majority were altogether devoid of processes.

As can readily be seen from the foregoing description, the cellular elements demonstrated in this tumor are not those usually found in glioma, but suggest a connective-tissue growth. Gliomata containing irregularly shaped giant cells have been described by Fleischl,* by Klebs, by Stroebe, and by myself, but in all these cases the majority of the cells in the tumor were either spider, brush, or the so-called ganglion cells, all of which were furnished with processes, and the intercellular substance was rich in fibres. An attempt to stain sections of this tumor by Golgi's method failed entirely, but this may have been due to the length of time which elapsed before the autopsy was made, and perhaps also to the long immersion in formaldehyde.

The tumor was unusually rich in blood-vessels of all sizes, from capillaries to large vessels with several layers of elastic and muscular fibres in their coats. The smaller vessels presented no peculiarity in appearance, but in the larger ones the outermost fibres, which composed the wall, could often be seen passing out into the tissue to lose themselves among the cells at a long distance from their origin (Plate XXX, Fig. 4). These fibres had all the characteristics of elastic tissue. They pursued a wavy course, were curled at the free ends, and did not branch. Again, in other places, fibres exactly like these in character were found running between the cells, though they could

* References to literature are at the end of this article.

not be traced to any vessel, and had no apparent connection with the cells of the tumor. By Van Gieson's stain they came out in sharp contrast to the cells, staining pink or deep red (Plate XXIX, Fig. 2); by Mallory's phosphotungstate they stained a purple red (Plate XXX, Fig. 3); but it was in sections treated with Weigert's fuchsin stain for elastic fibres that they could most easily be traced (Plate XXX, Fig. 4). By this stain they appeared bluish-black, and therefore quite different from the delicate intercellular fibrils and processes from the spindle cells, which by all of the above-mentioned methods took the usual protoplasmic stain. The staining properties of these fibres forbade the supposition that they could be remains of medullated nerves. Sections were stained by Weigert's differential method for myelin sheaths, with the result that the medullated nerves were found to end abruptly at the edge of the tumor, no trace of such a structure being demonstrable within the new tissue. This absence of medullated nerves is regarded by Stroebe as one proof that a tumor is not of nervous origin.

Much more striking, however, than the single scattered fibres, were the thick circumscribed collections of coarse and delicate fibres which appeared everywhere throughout the tumor. These were dense masses lying among and on top of the cells, being sometimes long and spreading, sometimes round. They appeared in every variety of shape, from symmetrical rosettes to long irregularly branching masses. The fibres which composed them were usually delicate, and curved slightly, sometimes thick and bristling. In the larger masses, and in almost all the circular ones, the centre was composed of a granular material, the fibres appearing clearly only at some distance from the centre (Plate XXX, Fig. 3). Under the low power it often looked as if the fibres were processes of the surrounding cells, and passed from them into the centre, an arrangement suggesting that of the tails of the spermatozoa in a seminiferous tubule of the testicle. A close examination, however, under the oil immersion failed to show that these fibres were connected with the cell bodies; they seemed simply to pass among and over the cells. The cells directly surrounding them were usually spindle-shaped, sometimes with, sometimes

without processes. When present, such processes took the protoplasmic stain.

Although no part of the tumor was free from these masses, yet it was noticeable that the larger the number of blood-vessels, the fewer the fibrillar masses. Many of them occurred in close connection with the vessel walls, either entirely surrounding a small vessel, or covering one side, or, what was most common, scattered at intervals along the course of a larger vessel. Occasionally a rosette would contain in its centre a small collection of cells (Plate XXIX, Fig. 2).

A close connection between the fibrillar masses and the vessel walls suggested the idea that this connection might be invariable, and that in cases where the masses seemed independent, they were lying along the wall of a vessel which had not been included in the section. To prove this, serial sections were made, but with the result that by far the larger number of these masses were without demonstrable connection with the vessels.

In their staining properties these fibrillar masses corresponded with the single scattered fibres already described, and thus produced a very striking appearance, especially in sections stained by Van Gieson's method, where the deep pink rosettes stood out in sharp contrast with the yellowish background. By Weigert's elastic-fibre stain it was often possible to follow the single scattered fibres and see them end in a rosette, and also in many cases to find such fibres running from the wall of the vessel to lose themselves in rosettes at varying distances from the vessel (Plate XXX, Fig. 4). Though the fibrils composing the rosettes were usually much more delicate than the diffuse fibres, and stained a little lighter, yet there seemed no room for doubt that the two were of the same origin and character.

I can find in the literature no description of collections of fibres such as these in the case under discussion. Gliomata rich in fibrils are of frequent occurrence, but in these the fibres are never arranged in circumscribed masses, nor do they take the differential stains for connective tissue. By the kindness of Dr. Flexner I was enabled to compare with this tumor sections from an ependymal-celled glioma described by him a year ago. The arrangement of the fibres in his case on

first sight resembled somewhat the arrangement in the one I am describing. Here, too, there are long and circular collections of fibres surrounded by cells, but these fibres are processes from the cells from which they pass to the vessel walls; they stain as do ordinary glia fibres, and the cells are of the type of ependymal cells. The two specimens cannot, therefore, be regarded as belonging to the same class of growths.

The question then resolves itself into the following: Is this a glioma with a peculiar form of sclerosis, or a fibrosarcoma with masses of elastic fibres in the matrix? There are several arguments in favor of the former alternative. In the first place, it is unusual to find a connective-tissue growth in the cerebrum which has not sprung from the meninges, or from the endothelium of the vessels. The possibility that the connective-tissue elements in the outer and middle coats of the vessels might proliferate and form a true sarcoma cannot be denied, but this is not the usual form of connective-tissue growths in the nervous system. Upon the assumption that the tumor is a glioma, the apparent dissociation of fibres and cells might be explained by considering that this tumor represents the adult stage of neuroglia, when, according to Weigert, the fibres no longer have connection with the cells. Taylor argues in favor of this view, and has given a description of two specimens of glioma, the one representing the embryonic stage, the other the adult stage of neuroglia. In the former the fibres of the matrix proceed from the cell bodies; in the latter they are entirely independent of the small round cells. Maximilian Herzog also follows Weigert's view as to the dissociation of glia cells and fibres, but modifies it by regarding the fibres as cells which have undergone a senile change, analogous to the cornification of epithelial cells. He described an ependymal cyst lined with cubical epithelial cells. The cells became slender bipolar spindles as they approached the edge of the cyst, refractive granules appeared in the protoplasm, and finally the free edge was covered with stiff slender fibres, staining like cornified material. He argues that in this case the neuroglia cells, being of epithelial origin, reverted to the original type and formed a true epithelial growth, but that the process here

was in all essentials analogous to the formation of the fibres of neuroglia by gradual flattening and cornification of the cells. He might, therefore, consider the collections of stiff fibres in my case as analogous to the cornification of the cells in the centres of epithelial pearls in carcinomatous growths.

On the other hand, the arguments in favor of the connective-tissue nature of the tumor are many. In gross appearance it differs from a typical glioma, for it is circumscribed, firm in consistence and easily enucleated, while a glioma usually infiltrates instead of displacing the normal tissue, and is soft in consistence. Again, the characters of the cells and of the intercellular substance do not suggest a growth springing from the neuroglia. The entire absence of medullated fibres is another point against glioma.

The strongest argument, however, in favor of the connective-tissue origin of the growth is based on the chemical nature of the fibres, as shown by their reaction to differential stains. The distinctive chemical characteristics of neuroglia fibres, as evinced by their affinity for certain stains, is especially emphasized by Weigert in his exhaustive work on human neuroglia, and is made by him the basis for determination as to what does and what does not belong to this tissue. It is much to be regretted that the tumor was not obtained in the fresh condition, so that it could have been stained by Weigert's method for neuroglia fibres, but in the absence of this test the reaction of the fibres to the three stains mentioned above (Van Gieson's, Mallory's phosphotungstate, Weigert's elastic-fibre stain) would seem to prove conclusively that they belong to the connective tissues. The origin of the growth must be from the connective-tissue elements in the walls of the blood-vessels, and these elements, instead of producing a fibrosarcoma of the usual type, have gone on to the production of polymorphous cells with aggregations of elastic fibres within the matrix. That this is unusual must be conceded, but there is certainly an analogue to such a process in the formation of islands of hyaline cartilage in chondrosarcoma, and of bone in osteosarcoma.

I am unable to find descriptions of fibrosarcomata in which the fibres of the matrix were proven to be elastic fibres. It would seem that the

question as to the exact nature of such intercellular fibres in these tumors has not been systematically studied. The application of Weigert's admirable stain for elastic fibres to the study of tumors and of other pathological conditions gives promise of yielding interesting results. Melnikow-Raswedenkow in a recent article has described the distribution of elastic fibres in various organs in normal and pathological conditions. He notes especially the richness in elastic fibres of the walls of blood-vessels, particularly of the adventitia, and finds that the elastic fibres in healthy and diseased organs and tissues are derived mainly from the vascular walls. Within the central nervous system elastic tissue is scanty and present only in the walls of blood-vessels. He states that in tumors no new formation of elastic tissue occurs, a statement, however, contradicted by the presence of a large amount of elastic tissue in the tumor now under consideration. There is no other apparent source for the elastic tissue found in the present tumor than that in the walls, especially the adventitia, of the blood-vessels, and this conclusion is in accord with Melnikow-Raswedenkow's observations concerning the normal distribution of this tissue in the brain as well as with the relationship, already described, of much of the elastic tissue to the vessel walls. Where this relationship is no longer apparent, its disappearance may be attributable to obliteration of vessels or to the severance of the original connections.

The literature on the normal development of elastic fibres is most unsatisfactory. There seem to be two theories as to the origin of these fibres—the cellular and the intercellular theory—dating from Theodor Schwann's belief in the transformation of the cells into fibres and Max Schultze's view that the cells fused to form the fibrillæ. B. Lwoff believes that the fibres are formed by the outer part of the protoplasm of the cells, which becomes differentiated and lies as a sheath around the inner protoplasm and nucleus.

The later observers, almost without exception, hold that the fibrils are formed in the intercellular matrix, independently of the cells, or at any rate without visible connection with them. Henle, Kölliker, Ranvier, Schaefer, and Minot uphold this view. According to Ranvier, there appear in the gelatinous matrix between the embryonic

connective-tissue cells globules of elastin, probably deposited by the cells. These fuse and form fibrils. They appear late in embryonic life (fifth month in human beings), grow by thickening, and continue to form even after birth. According to Minot, the elastic fibres in the omentum develop thus: The connective-tissue cells become long and spindle-shaped, with oval nuclei. In between them appear fibrils which increase in length and number, and gradually form bundles, which take a wavy course. Throughout their development they have no apparent connection with the cells. Schaefer has observed that in the development of cœlenterates fibres appear in the matrix at a period when there is entire absence of cellular elements.

As regards the mode of development of the elastic fibres in the outer coats of the blood-vessels, the literature is even more scanty. The description of the formation of new blood-vessels may be followed with precision until we pass from the capillary tube with its simple endothelial coat to the arteriole with a fibro-muscular coat, where the subject suddenly becomes obscure. Most works on embryology ignore completely the question of the origin of these fibres. Minot says they are formed by the differentiation of the surrounding mesenchyma, while the pathologists seem to consider that, so far as concerns the newly-formed vessels in proliferating tissues, the outer coats are formed by multiplication and differentiation of the endothelium of the capillary walls.

So long as the whole subject of the normal development of elastic fibres is unsettled, it would be useless to attempt to explain their occurrence in a pathological growth. Whether, however, they are formed from the bodies of cells, or deposited by the cells in the matrix, or arise independently, it is certainly conceivable that a growth, originating in the connective-tissue elements of the vessel walls, might produce, in an abnormal and irregular way, the same kinds of fibres as its prototypes have produced in the course of normal development. As said above, the process would seem to be somewhat analogous to the formation of islands of cartilage or bone in mixed sarcomata.

In the tumor under discussion the steps in the process of fibre formation seem to be similar to those cited above as described by

Minot in the normal development of elastic fibres in the omentum. Spindle cells, like those which he describes as appearing before the fibres are found, occur in bands through the tissue of the tumor and, though furnished with processes, have no apparent connection with the real elastic fibres which are found between them. They would seem, however, to be in some way concerned in the formation of these fibres as they are present in greatest numbers in the neighborhood of the rosettes and the masses of fibrils which lie along the vessel walls.

DESCRIPTION OF PLATES XXIX AND XXX.

Plate XXIX.

Fig. 1. Isolated tumor cells. Small, round and oval mononuclear cells. Small and large pyriform and fusiform cells. Giant cells with and without processes. One giant cell shows cellular inclusion. Leitz objective $\frac{1}{12}$ in. oil immersion, ocular 5. Mallory's phosphomolybdic acid hæmatoxylin.

Fig. 2. Typical field containing fibrillar masses of different shapes. One mass surrounds a small vessel almost completely. Two masses lie along the sides of vessels. One contains a group of cells in its centre. In one place scattered fibres pass between the tumor cells. Leitz objective $\frac{2}{3}$ in., ocular 3. Van Gieson's picro-acid-fuchsin.

Plate XXX.

Fig. 3. One medium-sized rosette. The fibres can be seen passing over the cells. Large giant cells form a group to one side. Leitz objective $\frac{1}{12}$ in., oil immersion, ocular 5. Mallory's phosphotungstic-acid hæmatoxylin.

Fig. 4. Two small rosettes near a blood-vessel. Elastic fibres can be seen passing among the tumor cells to the rosettes, also from the vessel walls to the rosettes. Most of the cells are large, round and oval, but at one side can be seen a band of spindle cells. Leitz objective $\frac{1}{12}$ in., oil immersion, ocular 3. Weigert's elastic-fibre stain. Nuclei stained with lithium carmine.

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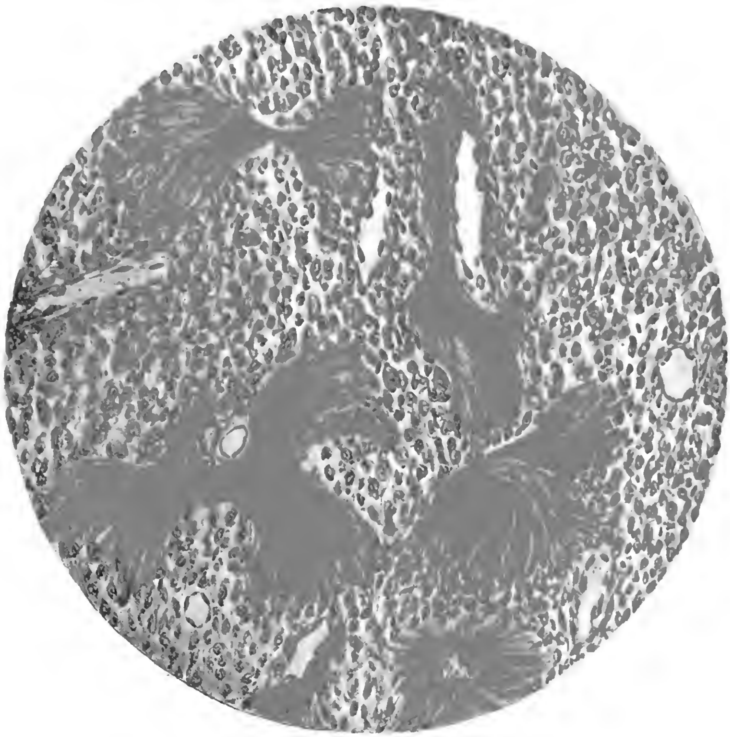

FIG. 2.

FIG. 1.

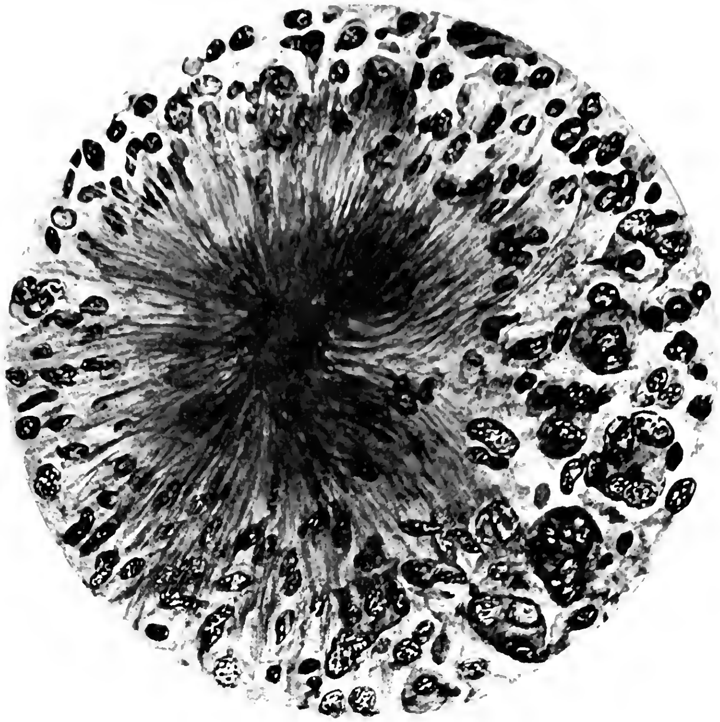

FIG. 3.

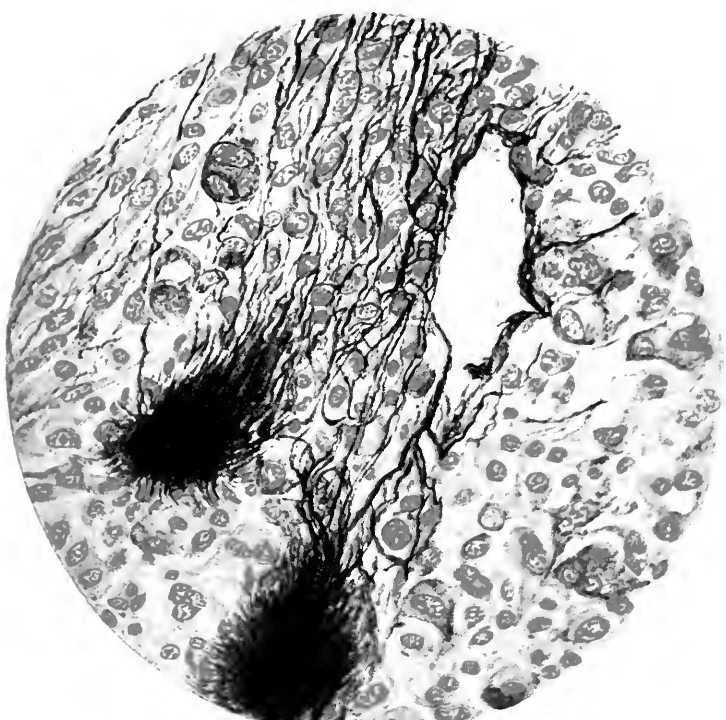

FIG. 4

ON THE DIFFERENTIATION AND CLASSIFICATION OF WATER BACTERIA.

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When bacteriologists have before them the problem of differentiation and classification of bacteria from water, independent of efforts to isolate with minimum delay some specific organism of disease, they proceed in a manner which is substantially as follows:

1. There is obtained on a given laboratory medium a pure culture of a special bacterium, the environment and life history of which, prior to its isolation at the laboratory, are for the most part unknown.

2. From the pure culture there are seeded a greater or less number of conventional culture media, known to differ in composition somewhat as prepared at different times at the same laboratory, and considerably as prepared by various workers in different laboratories.

3. These cultures are subjected at the laboratory to varying temperatures and periods of development leading up to final descriptions of cultural characteristics.

4. Observations upon and descriptions of the results obtained from the growth of this special bacterium under the above stated conditions are made.

5. Upon a comparison of the records of this bacterium with the limited descriptions of more or less similar forms given by other observers, it is found that they do not coincide, and therefore a new species is recorded.

Practically speaking, the above outline shows briefly and in a general way the conventional custom of bacteriologists in the past fifteen years, and which, as is well known, has brought the subject of species differentiation to a position which is untenable. Owing to the wide recognition of this state of affairs it seems to the writers to be futile to speak farther of the past.

It is the purpose of this paper to refer briefly to a number of points

associated with the above outlined procedure, and call attention to several modifications which experience, in a somewhat extended study of this subject, has led us to believe are of material assistance in the differentiation and classification of water bacteria.

I.—DEBILITATING ENVIRONMENT OF WATER BACTERIA BEFORE ISOLATION, AND THE CONSEQUENT DEGENERATION IN MANY INSTANCES AS NOTED AT THE LABORATORY.

Experience shows that in many cases two members of the same species of water bacterium, isolated at the same time and from the same sample, owing to divergent conditions in their earlier environments and in their life histories, will differ in some specific functions or characters, as studied in the laboratory. We have learned that, by transplanting successively such forms upon a series of media, these differences can, in a great measure, be eliminated. Accordingly, we have adopted the procedure of transferring the organisms after isolation from the pure culture on agar to nutrient broth; from the latter after three days' incubation at 20° C. to gelatine plates; and from a gelatine plate after the same period of incubation back to an agar tube again, from which the conventional media are seeded after the customary three days have elapsed. The value of this course, calculated to lend to the more or less debilitated form a rejuvenating action, and to bring the two members into a more nearly parallel physical condition before diagnostic tests are begun, is illustrated by the representative results given in Table I.

TABLE I.

EFFECT OF PRELIMINARY CULTIVATION ON BACTERIAL DEVELOPMENT.

Name of organism.	Test.	Result of Test.	
		Primary culture.	After preliminary cultivation.
Bacillus fluorescens liquefaciens.	Motility.	Negative.	Positive.
" " "	Nitrate reduction.	"	"
" prodigiosus.	Gas production.	"	"
" coli communis.	Coagulation of milk.	"	"
" ochraceus.	" "	"	"
" similtyphus.	Nitrate reduction.	"	"
" subtilis.	Motility.	"	"
" viridis.	Indol production.	"	"

In each case the temperature and period of development were 20° C. and 10 days, respectively, with the exception of the tests for motility, which feature was observed in cultures two days old.

Attention is particularly called to the last step in the process of preliminary cultivation, as it is important that, once having gotten an organism into a condition from which more favorable results may be obtained, the culture should be allowed to lose none of its regained vitality by being allowed to remain an unnecessarily long time on the agar tube before the various media are inoculated.

If the results of diagnostic tests are to be regarded seriously, and if

TABLE II.

EFFECT OF PRELIMINARY CULTIVATION ON BACTERIAL DEVELOPMENT.

Test.		Percentage constancy of results. Primary cultures.	After preliminary cultivation.
Bacillus—true form,		95	100
Motility,		87	100
Spores—heat test,		93	100
Growth at 37° C.,		98	100
Liquefaction of	Gelatine,	98	100
	Casein,	95	100
	Blood serum,	93	100
Fermentation of dextrose broth	Gas,	92	100
	Turbidity in closed arm,	89	100
Nitrate reduction,		90	100
Indol production,		97	100
Milk coagulated,		80	100
Fluorescence,		95	100
Chromogenesis,		100	100

such results are to lead to classifications which shall be of permanent value, it is obvious that such results must yield a high percentage of constancy. Otherwise they will be misleading and must be eventually discarded.

For the purpose of determining the percentage constancy of the results of prominent diagnostic tests, before and after transplanting the culture upon a series of media as described above, twenty cultures, representing eleven different species of water bacteria, were carried through a series of tests, the results of which appear in Table II.

Each of the media was seeded in triplicate with each culture from which both morphological and biological results were obtained. Development was continued for 10 days at 20° C. before the final results of the respective tests were recorded, although observations were taken day by day.

II.—ON THE COMPOSITION AND PREPARATION OF CULTURE MEDIA WITH ESPECIAL REFERENCE TO LIMITATIONS IN THE USE OF NUTRIENT CARBOHYDRATE SOLUTIONS AS PREPARED AT PRESENT.

In this connection the recommendations of the Bacteriological Committee* leave but little to be desired, so far as is practicable to be obtained at present. And in our species work during the past ten months, it has been our custom in the preparation of media to follow the procedures recommended, with no departure except a limitation in the use of the different carbohydrate solutions as now prepared.

In regard to the use of carbohydrate solutions prepared by present methods, our experience has shown an inconsistency in quantitative results in connection with gas production and the end reaction. In quite an extended study of the accuracy of these tests, the results obtained show the influence of three factors, intimately connected with the inconstant results that were often obtained, which are as follows:

1. The results of growth in a carbohydrate solution are affected somewhat by the possible degeneration of the organism.

2. The results of growth are often affected by the initial reaction of the carbohydrate solution.

3. Irregularities in the composition of the carbohydrate solution as prepared and sterilized, notably in the case of lactose and saccharose, also affect these results.

An illustration of the first factor is presented below in Table III showing representative results upon the quantity of gas and the end reaction in dextrose, lactose and saccharose broths, by six different cultures of Bacillus coli communis isolated on successive days from

* Procedures Recommended for the Study of Bacteria, *Journal of American Public Health Association*, Jan., 1898.

the Ohio River water. The temperature and period of incubation in all cases were 20° C., and ten days, respectively; and the cultures were not subjected to preliminary cultivation.

TABLE III.

REPRESENTATIVE RESULTS OF GROWTHS OF B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS.

Number of organism.	Carbohydrate.	Initial reaction 1.5 per cent Acid. Total gas (per cent).	End reaction (per cent).
1	Dextrose.	77	3.8
2		83	4.1
3		52	3.2
4		41	2.9
5		88	5.0
6		90	4.9
1	Lactose.	91	3.8
2		78	3.6
3		49	5.1
4		80	4.2
5		60	2.6
6		53	3.8
1	Saccharose.	48	3.0
2		0	2.7
3		13	1.9
4		15	0.8
5		2	1.0
6		2	1.6

From what has been stated with regard to the influence of preliminary cultivation, to overcome initial degeneration, as outlined in the last section, it would naturally be expected that such a procedure would eliminate in a measure the discrepancies in the above results. As shown in Table IV this preliminary step as carried out was appreciably helpful, but was incapable within the limits studied of making the quantity of gas of decisive diagnostic value.

Relative to the influence of preliminary cultivation upon the end reaction, it was found to be inadequate to bring about even fairly constant results, as shown in Table IV.

With regard to the second of the above factors it was found that within a fairly close range of initial reactions, as ordinarily employed and reaching from about + 1.5 to —1.5 per cent, its influence upon

TABLE IV.

GROWTH OF B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS, BEFORE AND AFTER PRELIMINARY CULTIVATION.

No. of Organism.	Carbohydrate.	INITIAL REACTIONS.											
		1.5 per cent. Acid.				Neutral.				1.5 per cent. Alkaline.			
		Before.		After.		Before.		After.		Before.		After.	
		Total gas (per cent).	End reaction (per cent)	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).
A	Dextrose	32	3.9	68	4.2	43	3.8	57	2.7	52	4.2	68	3.3
B	Dextrose	46	2.7	59	3.7	37	2.9	62	3.8	40	4.4	72	3.9
C	Dextrose	59	3.1	81	5.1	70	4.2	54	4.0	26	5.0	56	5.0
A	Lactose	62	4.2	62	2.9	39	3.4	52	3.3	47	2.9	62	2.9
B	Lactose	27	5.1	48	3.8	61	4.1	69	3.7	38	3.6	51	3.6
C	Lactose	82	3.7	74	3.9	58	3.8	69	4.1	59	2.7	63	3.9
A	Saccharose	10	1.9	83	4.7	12	3.3	64	3.8	0	—1.4	30	2.6
B	Saccharose	0	1.4	4	2.1	0	0.9	1	0.7	0	—1.2	0	1.4
C	Saccharose	0	1.6	1	1.7	0	0.6	1	0.6	0	—1.1	0	—1.4

the total amount of gas produced and the end reaction does not appear to be very marked. This is especially true if the period of incubation at 20° C. does not exceed 3 or 4 days. When the period of incubation reaches 10 days, then, even within the above stated limits, the initial reaction becomes a factor with reference to the final quantitative results.

But if the initial reaction should be more than about 1.5 per cent from the phenolphthalein neutral point, considerable variations in the results are found, as compared with those obtained with initial reactions within the stated limits. And in most cases it was noticeable that the quantity of gas was greatest in those tubes where the solution was initially most alkaline (—2.0 per cent). This is shown by the results presented in Table V (averages of more than 100 sets of results) with reference to B. coli communis freshly isolated from feces; and when the period and temperature of incubation were 10 days and 20° C. respectively.

TABLE V.

PERCENTAGE OF TOTAL GAS PRODUCTION BY B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS OF DIFFERENT INITIAL REACTIONS.

Initial reaction (per cent).	Carbohydrate.	
	Dextrose.	Lactose.
+2.0	35	28
+1.0	36	34
.0	45	45
—1.0	53	52
—2.0	68	64

In connection with the third factor mentioned at the outset of this section, it will be noted in the foregoing tables that the fermentation data from saccharose solutions, and to a certain degree from lactose solutions, are variable to the point of being erratic. Numerous experiments show them, however, to be representative even when obtained with the same culture. That is to say, there is decisive evidence to show that the variable results were due to the culture solutions and not to the bacteria themselves.

To explain fully these variations involves a thorough knowledge of the chemistry of carbohydrates, and is beyond the scope of this paper. It is sufficient to state that the explanation, in part at least,

appears to be associated with chemical changes (inversion, etc.) which are produced directly by the action of heat. Special efforts for several months were made to obviate the disturbing influence of heat, by using intermittent sterilization of the solution at low temperature, 70° C.; the use of sugars sterilized by dry heat at low temperature for long periods; and by means of adding the pure sugar dissolved in sterile water to the broth after the sterilization of the latter. None of these efforts were successful, and other work caused an abandonment of these studies.

Summing up in brief terms the experience recorded in this section, it may be stated that the quantity of gas formed in carbohydrate solutions and the end reaction of the solution are found to be too indefinite to be of value in the classification of bacteria; and that by the aid of dextrose alone better (more decisive) qualitative evidence is obtained than by the use of dextrose, lactose and saccharose.

III.—THE TEMPERATURE AND PERIOD OF DEVELOPMENT OF WATER BACTERIA DURING CULTIVATION FOR CLASSIFICATION TESTS.

Temperature of Development.—It is undoubtedly true that, for the purposes of prompt differentiation of bacteria intimately associated with the causation of disease, and for detailed comparative studies of races or varieties of a given species, it is necessary that there shall be made comparisons of the results of growths of cultures at substantially 20° and 37° C.; yet, for the classification of water bacteria, it is our experience that cultivation at 20° for each set of tests is sufficient. Of course, it must be learned whether or not the bacterium will grow at 37° C.; and this, according to our present custom, is obtained by means of an agar plate culture.

In explanation of our departure from the custom of employing both 20° and 37° C. for temperatures of development, it is to be stated that such was our practice, in strict accord with the recommendations of the Bacteriological Committee, during the early part of our work at this laboratory. But soon we became satisfied that, for the purposes of classification of water bacteria, the increase in amount of definite information obtained from cultures grown at 37° C. was slight, and to our thought clearly incommensurate with the labor involved. Further-

more, there are substantial grounds for believing that at times the data obtained from the cultivation of water bacteria at 37° C. are misleading, owing to inability by ready means to distinguish uniformly between negative results from positive growths and negative results due to the absence of growth caused by the high temperature. Such data, even if occurring at fairly rare intervals, can lead ultimately only to serious confusion.

Period of Development.—This portion of bacterial procedures has not been standardized to the degree which seems to be imperative for those workers who have in view the object stated in the introduction of this paper. A standard period of development involves the consideration of the following factors:

1. It should not be so short as to exclude a considerable portion of the definiteness of the recorded characters of the given bacterium.

2. It should not be so long as to add unnecessarily to the tediousness of the methods and to difficulties in their applications to practical problems of medical and sanitary science; nor increase the likelihood of complications arising from contamination of the culture or the evaporation of the medium; nor force the constant use of rubber caps to protect the contents of the tubes, as it is found that such a practice affects some growths through the exclusion of oxygen.

3. It must of necessity be an arbitrary limit, lying between the wide extremes now employed by various workers, and filling in a conservative manner an intermediate position with reference to the two factors above stated.

At the outset of our present studies, it was the custom to keep all cultures for four weeks before the final descriptions and tests were made. It was learned, however, after twenty bacteria had been carefully studied, that substantially no changes of specific value were ordinarily obtained after the tenth day; and it was not until this period had elapsed that satisfactory data in several instances could be obtained upon the liquefaction of gelatine, a test which occupies a prominent place in the present bacterial methods.

Accordingly, it was decided to adopt ten days as the standard period of development in our work, and disregard in our classifications any change which by chance might be noted beyond that time, should

the cultures be preserved. It is not to be supposed that the writers are unmindful of the fact that in some instances well-marked changes may take place after the tenth day of cultivation, notably with reference to chemical changes associated with fermentation processes as in the case of milk; but it is our position that under such conditions changes of differential value are seldom obtained, and we are not yet ready to accept that they deal with species rather than with races of bacteria, in the present state of bacterial classification.

IV.—PROCEDURES DIRECTED TO FULFILL THE NECESSITY OF ARRANGING WATER BACTERIA IN GROUPS AS A PRELIMINARY STEP TOWARDS CLASSIFICATION.

Irresistibly bacteriologists working in this field have been drawn towards efforts having for their purpose the arrangement of fairly similar forms into subdivisions, which facilitate the comparative study of closely allied species or races of bacteria. Various workers have referred to their efforts in this direction as "groups," "synopses," "summaries" or "classes" of the bacteria which they studied. The importance of such steps, which has been set forth by a number of writers in the past few years, is probably conceded by substantially all workers in this line. Recently, the time-consuming task of arranging, so far as practicable, the recorded species of bacteria to date into twenty-five classes was completed by Chester* and his contribution has proven to be very helpful to us.

Many bacteriologists in the past, if not at the present time, have been inclined to move with much slowness in efforts to arrange bacteria into groups, on the ground that they could not see their way clear towards a *natural* grouping. It is of course true that with present methods of bacteriology all schemes of grouping bacteria are purely arbitrary; but it is also true that there is no probability of any one ever devising a bacterial grouping which is natural; or, in the immediate present, one which will be permanent and acceptable to all workers.

The arrangement of bacteria into groups is wholly a matter of con-

* A preliminary arrangement of the species of the Genus Bacterium by Frederick D. Chester. From Report of the Delaware College Agricultural Experiment Station, 1897.

venience, undertaken to assist in making comparative studies of similar forms and of data related thereto. While it is true that each bacteriologist might have a different method of grouping, which in his own hands was satisfactory for the purpose to which it was put, and therefore successful, yet it is obvious that a grouping used by many workers in common would lead to more systematic results and more rapid improvement of current methods.

According to our experience the present methods of grouping water bacteria are not wholly satisfactory for the following reasons:

1. The groups are too many in number, and depend for their separation upon results consequent upon immediate environment, and not upon inherent characteristics of a specific nature.

2. Following the views of biologists in other fields the importance attached to morphological data is greater than justifiable, and these data are used to the exclusion of results of more definite physiological tests.

In connection with the second of these points, it may be stated that we have had considerable difficulty in deciding uniformly as to the form (under apparently all ordinary conditions) of those very plump bacilli which at one time were specified as bacteria, and which could quite properly be classed as micrococci. Such forms are quite prevalent in some waters, although they seem to include only a very few species. With regard to spore formation and motility, we feel quite sure of our data upon these points when we make use of our regular custom of applying the heat test for spores, and for motility when we compare preparations in hanging drops of water and of formaldehyde.

The following tests are used at this laboratory to obtain data for the separation of water bacteria into groups to aid in their study and classification.

1. Fluorescence and chromogenesis.
2. Liquefaction of gelatine.
3. Well-marked characteristics of typical gelatine plates.
4. Fermentation of carbohydrates.

By means of the preliminary cultivation methods described in Section I of this paper, the preparation of media as referred to in Section II, and the standard temperature and period of development as outlined in Section III, the writers have found that from the four

tests above stated a practically constant arrangement of water bacteria may be obtained as indicated by the following outline:

TABLE VI.

DATA FOR ARRANGEMENT OF WATER BACTERIA IN GROUPS.

- Water Bacteria.
  - Fluorescent.
  - Non-Fluorescent.
    - Chromogenic.
      - Red, Orange, Yellow, Violet.
    - Non-Chromogenic.
      - Gelatine liquefied.
        - Characteristic colonies on gelatine plates.
          - Proteus form.
          - Subtilis form.
        - Non-Characteristic colonies.
          - Fermentation of carbohydrate.
            - Gas production.
            - No gas production.
          - Non-Fermentation.
      - Gelatine not liquefied.
        - Fermentation of carbohydrate.
          - Gas production.
          - No gas production.
        - Non-Fermentation.

From the above schedule thirteen groups of water bacteria are obtained as follows:

Group I. All fluorescent forms.

Group II. All red chromogenic forms.

Group III. All orange chromogenic forms.

Group IV. All yellow chromogenic forms.

Group V. All violet chromogenic forms.

Group VII. All non-fluorescent, non-chromogenic, gelatine-liquefying bacteria, forming proteus-like colonies on gelatine.

Group VII. All non-fluorescent, non-chromogenic, gelatine-liquefying bacteria, forming subtilis-like colonies on gelatine.

Group VIII. All non-fluorescent, non-chromogenic, non-proteus- and non-subtilis-like bacteria, which liquefy gelatine and ferment carbohydrate with the production of gas.

Group IX. All bacteria conforming to the specified characteristics of Group VIII, except that fermentation of carbohydrate takes place without the formation of gas.

Group X. All bacteria conforming to the specified characteristics of Group VIII, except that no fermentation of carbohydrate occurs.

Group XI. All non-fluorescent, non-chromogenic, non-gelatine-liquefying bacteria, which ferment carbohydrates with the production of gas.

Group XII. All bacteria conforming to the specified characteristics of Group XI, except that fermentation of carbohydrate takes place without the production of gas.

Group XIII. All bacteria conforming to the specified characteristics of Group XI, except that no fermentation of carbohydrate occurs.

The features upon which the above groups are based we are led to consider, arbitrarily, as "fixed characters." Being looked upon as such, it becomes necessary that certain provisions shall be observed in their consideration in order that the features true of one group may not become diffused into the one closest allied. Chromogenesis, for instance, upon which the separation of four of the above groups are based, rarely appears the same upon two different media owing mainly to differences in their composition. For obvious reasons we have

deemed it advisable to adopt a specific medium (or media) for a given observation and the conditions governing the chief features of the first five groups are therefore briefly given, as follows:

Group I.—Fluorescence should be observed exclusively in agar tube cultures; and, since a given reaction will not under all conditions be found applicable to the production of pigment for all bacteria, it is necessary that agar of three reactions should be used in this test, namely, 1.5 per cent acid, neutral, and 1.5 per cent alkaline, to phenolphthalein.

Groups II, III, IV, and *V.*—The hues or shades of color produced by the growth of certain bacteria upon culture media are well understood to be admixtures of certain colors. Most prominent among these mixed colors is that of yellow and its modifications. While the reds and violets are readily handled, it will require some study to deal effectually with the yellow and orange chromogens. We have learned that colors which are neither yellow nor orange must be set aside and placed among the non-chromogenic forms. Brown and grey-yellow colors frequently observed are instances of this character.

We have found that the most constant results in the study of chromogenic bacteria are obtained from agar tube cultures.

The colors embraced in the above four groups may be briefly explained as follows:

Red.—That color produced by B. prodigiosus on agar.

Yellow.—That color produced by the growth of Sarcina lutea on agar with the dividing line between this and the orange group at the yellow-ochre hue produced by the growth of B. ochraceus on agar.

Orange.—This color begins just below the yellow ochre. The true orange color is the same as that produced by the growth of B. aurantiacus on agar.

Violet.—The same as that color produced by the growth of B. violaceus on agar.

V.—ON THE NECESSITY OF EMPLOYING FOR PURPOSES OF CLASSIFICATION OF WATER BACTERIA DATA OF DEFINITE (POSITIVE OR NEGATIVE) INFORMATION, AND THE EXCLUSION OF THOSE DATA WHICH ARE NOT SHARPLY DEFINED TO A UNIFORM DEGREE.

Upon studying the literature of the differentiation of water bacteria, it is readily apparent to practically all bacteriologists, as was brought out in the instructive paper by Dr. Wyatt Johnston,* that the data now available upon this subject are weak and lacking in two notable ways:

1. Too many of the data, to which a differential value is given, are so indefinite that there is no assurance that the observations could be regularly duplicated even by the original worker; and, accordingly, the records of such work when published not only fail to advance the subject in a substantial manner, but even complicate and confuse matters to a serious degree.

2. There are too few data which may be called definite and which all experienced workers with good technique have reasonable expectations of confirming uniformly.

Among the principal objects of the work of the Bacteriological Committee in setting forth standard procedures was, by the use of uniform and improved methods, to increase the number of definite data by the elimination of certain ones from the first of the above classes and their transposition to the second class.

To what degree these efforts will meet with success can of course be told only after the experience of a large number of workers has been recorded, discussed and brought to a fairly agreeing consensus of opinion.

As a result of our experience of the past ten months in applying the procedures of the Committee, the writers find that in that time their views have become somewhat modified. We have become impressed with the absolute necessity of placing the subject of the classification of water bacteria upon a basis such that the systematization

* On Grouping Water Bacteria, *Journal Amer. Public Health Assn.*, Oct., 1895. p. 445.

shall have for a foundation only such data as can be readily confirmed by all capable workers employing standard methods. Or, expressed in the terms of this paper, there must be secured a foundation for future work that is made up of the results of tests which have substantially 100 for a percentage constancy, when the tests follow promptly a course of preliminary treatment to eliminate the influence of possible initial degeneration.

To some it will occur that such a decision may lead to a classification which would be very crude and fragmentary. In a measure that may be true, but to our thought the procedures of the Committee insure sufficient tests of a percentage constancy of practically 100 to lead to a fairly satisfactory set of data for primary classification, and that the advantage of all bacteriologists having a definitely located datum point in this work far outweighs at present the objection of crudeness.

The future of those tests which from our present evidence have a percentage constancy materially less than 100 is one of much uncertainty. Some of the tests will probably have their technique sufficiently improved to be elevated to the class yielding uniformly definite data. Others will probably have a field of greatest usefulness, after more or less modification in technique, in connection with the prompt identification of disease germs, and studies in the separation of species and varieties, and in tracing the life history of bacteria under different environments and from a purely scientific standpoint. To condemn them for all time seems unjustifiable at present, especially as other workers under other conditions may obtain different results.

With reference to those tests which in our hands at present seem to yield indefinite results (with a percentage constancy of much less than 100) we have no further comment to offer at this time; and list the more important ones below with the statement that from the results of our experience we are not ready to accept them as yielding data of sufficient definiteness to allow them uniformly a place at present in the classification of water bacteria. In every instance in the following list color is regarded as independent of fluorescence and chromogenesis.

LIST OF TESTS RECOMMENDED BY THE BACTERIOLOGICAL COMMITTEE WHICH HAVE BEEN FOUND TO YIELD LESS THAN 100 FOR A PERCENTAGE CONSTANCY.

MORPHOLOGY.

Capsulation. Vacuoles. Staining of spores. Presence of crystals within the cells. Pleomorphism.

BIOLOGY.

Gelatine Plate.—Size and color of colonies and growth under the mica plate.

Agar Plate.—Size, color and margin of colony.

Gelatine Tube.—Color of growth.

Agar Tube.—Extent, shape, margin and surface relief of growth.

Nutrient Broth Tube.—Time elapsing before the appearance of turbidity, deposit and surface pellicle. Color of broth. Appearance after shaking. Color, thickness and structure of pellicle. Color and amount of deposit. Quantitative reaction after a stated period.

Milk.—Color of milk. Formation of gas. Amount of whey. Quantitative reaction.

Fermentation Test. Quantitative.—Reaction of solution. Quantity of gas. Differential value of different sugars.

Lactose Litmus Agar.—Change of color.

Potato.—Color, restriction, shiny appearance and lustre of growth; and formation of gas.

VI.—THE ARRANGEMENT OF DEFINITE DIFFERENTIAL DATA IN THE FORM OF A CHART, AS A MATTER OF CONVENIENCE IN THE CLASSIFICATION OF WATER BACTERIA.

Excluding from the total number of differential tests appearing in the report of the Committee, those which were listed in the last section as insufficiently definite and constant in their value for present purposes, there remain the data from 26 tests. In the light of our experience these results can be recorded as positive or negative, with little or no material loss as to explicitness and with a decided gain as to convenience and uniformity of expression. From our studies of the

literature upon this subject we are convinced that the latter point is no small one, owing to the great tendency of the observer to associate his point of view and his style as a writer with his text descriptions.

The method of recording the results of the leading tests in positive or negative terms allows, of course, the use of plus or minus signs. Early in the work at the laboratory a chart was prepared showing the results of the species work as it advanced, and permitting the main bulk of the work to be presented in a concise and convenient manner. The heading and arrangement of this chart, together with the records of the differential characters of 42 species of bacteria isolated from the Ohio River water at Cincinnati, are presented at the end of this article. The chart presents in explicit terms the principal records of each species isolated. It also makes simple the task of showing wherein similar species differ from each other. Furthermore, by preparing a chart showing the record of each distant species met with, it is easy to note whether or not any species subsequently found agrees with others previously studied. By making the current records on a chart of the same size and folding it so that the records of the given form under study appears on the top line, a glance of the eye is sufficient to show with which species it is identical, if with any.

In connection with this chart, which is of much assistance in classifying water bacteria according to the reliable methods now available, it of course is not to be understood that it necessarily would serve that purpose as the subject advances, especially as consideration is given to questions of races versus species. It is possible, however, that it may be amplified to serve that purpose for some time.

March, 1899.

CHART.—CLASSIFICATION OF BACTERIA F

NAME OF ORGANISM.	FIRST INVESTIGATOR.	MORPHOLOGY.				CULTUR				
						Nutrient broth tube.		Nutrient agar tube.		Ca a
		Bacillus.	Diameter greater than 1μ.	Motile.	Spores.	Scum.	Turbidity.	Dull.	Wrinkled.	
						Group I. Fo				
B. fluorescens liquefaciens	Flügge	+	—	+	—	+	+	—	—	+
B. fluorescens non-liquefaciens	Eisenberg	+	—	—	—	+	+	—	—	—
B. viridis	Lesage	+	—	+	—	+	+	—	—	+
B. fluorescens ovalis	Ravenel	+	—	+	—	+	+	—	—	—
B. pyocyaneus	Gessard	+	—	+	—	+	+	—	—	+
B. fluorescens incognitus	Wright	+	—	+	—	+	+	—	—	—
						Group II. Chro				
B. prodigiosus	Ehrenberg	+	—	+	—	+	+	—	—	+
B. rubidus	Eisenberg	+	—	+	—	+	+	—	—	+
						Group III. Chro				
B. arborescens	Frankland	+	—	+	—	—	+	—	—	+
B. aurescens	Ravenel	+	—	+	—	—	+	—	—	—
B. fulvus	Zimmermann	+	—	—	—	—	+	—	+	[illegible]
B. fuscus	Zimmermann	+	—	—	—	—	+	—	—	[illegible]
B. aurantiacus	Frankland	+	—	+	—	—	+	—	—	+
						Group IV. Chron				
B. desidiosus	Wright	+	—	—	—	+	+	—	—	—
B. ochraceus	Zimmermann	+	+	+	+	—	—	—	+	—
B. flavescens	Pohl	+	—	+	—	—	+	—	—	—
B. lactis erythrogenes	Hueppe	+	—	—	—	+	+	—	+	+
B. subflavus	Zimmermann	+	—	+	—	—	+	—	—	—
Sarcina lutea	Schroeter	—	—	—	—	—	—	—	—	+
						Group V. Chron				
B. janthinus	Zopf	+	—	+	—	+	+	—	+	+
B. violaceus	Frankland	+	—	+	—	+	+	—	—	+
						Group VI.				
B. mycoides	Flügge	+	+	+	+	+	—	+	—	+
B. mesentericus vulgatus	Eisenberg	+	+	+	+	+	+	—	+	+
B. proteus fluorescens	Jäger	+	—	+	—	+	+	+	—	+
						Group VII. S				
B. subtilis	Ehrenberg	+	+	+	+	+	—	+	+	+
B. cereus	Frankland	+	+	+	+	+	+	+	+	+
						Group VIII				
B. cloacæ	Jordan	+	—	+	—	+	+	—	—	—
B. liquefaciens	Eisenberg	+	—	—	—	—	+	—	—	—
						Group IX.				
B. liquidus	Frankland	+	—	+	—	—	—	—	—	—
B. antenniformis	Ravenel	+	—	+	—	+	—	—	—	—
						Group X. S				
B. superficialis	Jordan	+	—	+	—	+	+	—	—	—
B. annulatus	Wright	+	—	+	—	—	+	—	—	—
B. flexuosus	Wright	+	—	—	—	+	+	—	—	—
B. geniculatus	Wright	+	—	+	—	+	+	—	—	—
B. aquatilis communis	Zimmermann	+	—	+	—	+	+	—	—	—
						Group XI				
B. coli communis	Escherich	+	—	+	—	+	+	—	—	—
B. aërogenes	Escherich	+	—	—	—	+	+	—	—	—
						Group XII.				
B. similtyphus	....	+	—	+	—	+	+	—	—	—
B. solitarius	Ravenel	+	—	+	—	+	+	—	—	—
B. aquatilis sulcatus I	Weichselbaum	+	—	+	—	—	+	—	—	—
B. aquatilis sulcatus V	Weichselbaum	+	—	+	—	—	+	—	—	—
						Group XIII.				
B. candicans	Frankland	+	—	+	—	—	+	—	—	—

BIOLOGY.

	Potato tube.	Fermentation tube.	Biochemical Features.													Pathogenesis.
						Liquefaction.			Gas production.					Nutrient agar tubes.		Mice.
	Luxuriant.	Growth in closed arm.	Grows at body temperature.	Facultative anaerobe.	Affected by range of reaction	Gelatine.	Casein.	Blood serum.	Dextrose broth.	Nitrate reduced.	Indol produced.	Milk coagulated.	Fecal odor.	Chromogenesis.	Fluorescence.	Intra-peritoneal inoculation.
...Type.																
	−	−	−	−	+	+	+	+	−	+	−	+	−	−	+	−
	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−
	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+
	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−
	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+
	+	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−
...ype.—Red.																
	+	+	+	+	+	+	+	+	+	+	−	+	+	crimson	−	−
	−	+	−	+	+	+	−	−	−	−	−	−	−	brick red	−	−
...pe.—Orange.																
	+	+	−	+	+	+	−	+	−	−	−	+	−	orange	−	−
	−	−	+	−	+	+	+	+	−	−	−	+	−	"	−	−
	−	+	+	+	+	+	−	−	−	−	−	−	−	"	−	−
	−	+	+	+	+	−	−	−	−	−	−	−	−	"	−	−
	−	−	−	−	+	−	−	−	−	+	−	−	−	"	−	−
...pe.—Yellow.																
	+	−	−	−	+	+	+	+	−	−	−	+	−	yellow	−	−
	−	−	+	−	+	+	−	+	−	+	−	+	−	"	−	−
	+	+	−	+	+	−	−	−	−	+	−	−	−	"	−	−
	+	−	−	−	+	+	+	+	−	+	−	+	−	"	pink	−
	−	−	+	−	+	−	−	−	−	+	−	−	−	"	−	−
	−	+	+	+	+	+	−	−	−	−	−	−	−	"	−	−
...pe.—Violet.																
	+	−	−	−	+	+	+	+	−	+	−	−	−	dark violet	−	−
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BACILLUS PYOCYANEUS AND ITS PIGMENTS.

By EDWIN O. JORDAN, Ph. D.
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The blue or blue-green stains that sometimes appear upon surgical dressings long ago attracted the attention of observers and, even before the cause of the phenomenon had been discovered, Fordos* carried out some important investigations upon the nature of the coloring substance. In 1882 Gessard† proved that the color is produced by a bacillus (B. pyocyaneus) which he was able to isolate in pure culture, and whose morphological and physiological characters he carefully described. Since Gessard's discovery, B. pyocyaneus has been frequently subjected to the crucible of experiment and its biological peculiarities have been scrutinized with a degree of minuteness not yet exercised in the study of most microorganisms.

Attempts to determine the nature of the pigment, and the conditions under which the chromogenic property is manifested have not, however, led to concordant results. At one extreme we find an investigator‡ who maintains that only one pigment is formed by the different "races" or "varieties," and that this pigment is identical with the ordinary blue-green fluorescent substance produced by the growth of a number of common water bacteria, and who also is of opinion that the several "races" differ chiefly in their power to produce ammonia, a substance that, like other alkalies, modifies the color of the fluorescent pigment; at the other extreme we find observers§ who claim that one and the same variety of B. pyocyaneus is able to produce as many as four distinct pigments, black, blue, green and

* *Compt. rend. Acad. des sciences.* 1860, li, p. 215.

† La pyocyanine, Thèse. Paris, 1882.

‡ Thumm, Beiträge zur Biologie der fluorescierenden Bakterien, *Arb. a. d. Bact. Inst. d. techn. Hochschule zu Karlsruhe*, 1895, i, 291.

§ Charrin and de Nittis, *Compt. rend. Soc. de biol.*, 1898, 10. s., v, 721.

yellow, at the same time and in the same culture medium; while between these extreme positions are found some investigators who hold to the existence of different races or varieties of B. pyocyaneus, each race being characterized by the ability to produce a different pigment or pigments; there are also some who maintain that by varying the nutrient substratum one and the same race may be compelled to form differently colored metabolic products.

In the existing chaotic condition of bacterial classification and description, it behooves all investigators to move circumspectly in the matter of identifying the "species" or "races" with which they are working, and in the present instance I have thought it desirable to describe with some fulness the cultures that I have employed.*

I have made use in all of seven cultures. All these have certain characteristics in common: The bacteria are small bacilli with rounded ends, and for the most part average from 0.3μ-0.5μ in breadth by 1μ-2μ in length, but there is considerable variation upon different media, and these dimensions are frequently exceeded in one and the same culture. They are sometimes grouped in short chains, but are usually single. All are actively motile. Spores have never been observed. The bacilli stain readily with the ordinary aniline dyes, but lose the stain when treated by Gram's method. Growth under the mica plate, when it occurs at all, is very scanty, and none of the cultures produces pigment in the absence of oxygen. All grow more luxuriantly at 37.5° C. than at the room temperature. Gelatin is liquefied by all. The origin of the various cultures was as follows: Four, designated respectively as "B. pyocyaneus α, Gessard," "B. pyocyaneus β, Ernst," "B. pyocyaneus pericarditidis" and "B. pyocyaneus γ, Freudenreich," were sent to me from Kral's collection; one ("B. pyocyaneus, Mich.")† was sent to me through the kindness of Dr. Novy of Ann Arbor; one ("B. pyocyaneus, Albany") was obtained at an autopsy "from a focus of broncho-pneumonia" (Jan., 1899) and was sent to me through the courtesy of Dr. George Blumer

* I am indebted to Mr. H. E. Davies and Mr. E. E. Irons for assistance in the study of these cultures.

† Dr. Novy informs me that the parent culture was brought by him from the Berlin Hygienic Institute in 1888.

of the Bender Hygienic Laboratory; and one ("B. pyocyaneus, Rush") was furnished me through the kindness of Mr. Danielson of the Rush Medical College and was originally obtained (March, 1898) from the body of a guinea-pig that had died after inoculation with a fragment of diphtheritic membrane.

These cultures, while agreeing in certain points, showed among themselves differences which, though in some respects trivial, have proved fairly constant and characteristic. All the cultures, except B. pyocyaneus, Albany, have been in my possession for at least a year. Save where otherwise indicated, I have employed the methods recommended by the Bacteriological Committee.*

As regards morphological characters, I have been able to detect few uniform or significant differences. Variations in dimensions and grouping similar in character and extent to those commonly displayed among the varieties of the colon bacillus have, however, been observed. For example, B. pyocyaneus, Albany, when grown on potato, develops larger forms and shows a greater tendency to form chains than the other varieties. B. pyocyaneus γ displays a tendency both in broth and on potato to form short chains, and takes the stain more evenly than in the other cultures. B. pyocyaneus β is, on the average, not so large as the other bacilli.

I have observed no noteworthy differences in respect to behavior toward stains. All the cultures stain somewhat irregularly when treated with Löffler's methylene-blue, a point I have not seen mentioned by other observers. None of the seven cultures hold the stain when treated by Gram's method; on this head my observations accord completely with those of Růžička.†

Gelatin.—In the growth upon gelatin plates I have been able to discover no salient and constant differences in either the macroscopic or microscopic appearances of the colonies, although differences in the rapidity of growth and of liquefaction can be noticed. Divergences in growth upon gelatin are best seen in stab-cultures. The most rapid liquefaction occurs with B. pyocyaneus α, but B. pyocya-

* *Journ. Amer. Pub. Health Assoc.*, Jan., 1898.

† *Archiv f. Hyg.*, 1899, xxxiv, p. 153.

neus β, pericarditidis, and Albany are not far behind. B. pyocyaneus, Rush, grows at first somewhat more slowly, but in about 7 days overtakes the others and in 15 days outstrips them all. B. pyocyaneus, Mich., always lags behind the five already mentioned and the rear is brought up by B. pyocyaneus γ, which liquefies the gelatin most tardily. In all the cultures except B. pyocyaneus γ the liquefaction is at first superficial, a shallow, saucer-shaped depression forming slowly and then gradually extending to the sides of the tube. The liquefied gelatin, which is more or less colored, is sharply separated by a horizontal line from the unaltered medium below; some growth takes place along the inoculation line, but at first little or no liquefaction. Later, however, considerable liquefaction occurs along the inoculation line in B. pyocyaneus, Rush, Albany and β. The growth of B. pyocyaneus γ is of the stocking-shaped variety from the start, and even after 15 days' liquefaction has not reached the walls of the test-tube. In all a pellicle is formed on the surface of the liquefied gelatin; this is most marked in the case of B. pyocyaneus, Mich.

Agar.—Upon sloped agar there are few important divergences. The features most worthy of note are shown by B. pyocyaneus, Rush; this culture forms a very thin, delicate film which rapidly covers the whole surface and has a pronounced metallic lustre. In the other cultures the surface growth, which is projecting, is of a dull yellowish-white.

Broth.—In ordinary peptonized meat broth of the standard reaction, kept at 37.5° C., B. pyocyaneus, Rush, is the first to produce a pigment. The broth is rendered turbid by all the species and a heavy flocculent sediment is formed; a surface pellicle is produced by all, but is more tenacious in the cultures of B. pyocyaneus, Mich., than in the others.

Potato.—Interesting differences appear in the growth upon potato. The growth is luxuriant, dry, projecting and of a chocolate brown color in four cases, viz.: B. pyocyaneus, Mich., γ, β, and pericarditidis. The growth produced by B. pyocyaneus α is moist and glistening, but in other respects resembles that of the four cultures already mentioned. The potato itself is not discolored by these five species.

B. pyocyaneus, Rush, however, colors the potato a beautiful deep blue and B. pyocyaneus, Albany, imparts to it a greenish hue, the color in the latter case developing less rapidly and never becoming so intense as in the former. The growth of B. pyocyaneus, Rush, is more spreading and less projecting than the others.

The "chameleon phenomenon," as it was termed by P. Ernst,* is manifested by B. pyocyaneus, Rush, but by none of the others. The chameleon phenomenon, it will be remembered, consists in the change of color observed when the growth upon potato is touched with a platinum needle. It depends doubtless on the well-known fact that a substance is produced by the growth of the bacillus which by contact with the air is oxidized to the blue pigment known as pyocyanin. The shifting play of tints seen when a colony is touched is due to the conflicting action of the atmospheric oxygen and of the reducing substances doubtless present among the metabolic products of the bacillus. The exposure of the pyocyanigenic substance to the air affords an opportunity for the development of the blue color. I may here anticipate the remainder of this paper so far as to state that my observations indicate that the reason for the appearance of the chameleon phenomenon in some races of B. pyocyaneus and not in others lies in the varying degree in which pyocyanin is produced. Those races that produce pyocyanin vigorously and abundantly manifest the "chameleon phenomenon;" others, in which the pyocyanigenic function is weaker or altogether absent, are unable to display this peculiarity.

Indol.—Only two of the cultures possess the power of forming indol in any quantity in Smith broth; these are B. pyocyaneus, Rush, and B. pyocyaneus, Mich., the former showing itself much the more vigorous in this particular. B. pyocyaneus, Albany, produced a very slight amount of indol and the other four never gave a positive reaction. An attempt was made to accentuate or develop the indol-forming power by passing the organism through a succession of peptone cultures according to the method suggested by Peckham,† but

* *Zeitschr. f. Hyg.*, ii, p. 369.
† *Journal of Experimental Medicine*, 1897, ii, p. 549.

without avail so far as the awakening of any latent indol-forming power was concerned. A few such transfers, however, exalted perceptibly the indolfacient power of B. pyocyaneus, Rush, and to a lesser degree that of B. pyocyaneus, Mich. Freshly isolated cultures of B. pyocyaneus are, in general, recorded as yielding a positive indol reaction.*

Milk.—All the cultures curdled litmus milk at 37.5° in about 2 days. B. pyocyaneus γ, however, was usually 24-36 hours behind the other cultures. The color of the litmus was discharged, but on testing the reaction of the milk at the expiration of 2 days, and again after 10 days, it was found alkaline in all cases. When the cultures were allowed to stand the casein was slowly digested. Control tubes showed no change. At the room temperature the action upon milk was similar, but less rapid than in the thermostat, curdling not beginning until after the lapse of 5 or 6 days.

Reduction of Nitrates.—In nitrate broth B. pyocyaneus, Rush, reduced nitrates to nitrites quickly and completely; B. pyocyaneus α showed a much slighter reducing power, and the other five cultures gave a negative reaction. Severin† observed active reduction of nitrates in a culture of B. pyocyaneus recently isolated by himself from horse-dung. Dyar‡ observed reduction of nitrates in a culture of "B. pyocyaneus" from the laboratory collection and found also that a culture of "B. fl. liquefaciens" reduced nitrates well, but not completely; while another culture found in the exudate from a sick lepidopterous larva resembled the latter in all respects except in not reducing nitrates. Thumm§ and Růžička,‖ two of the investigators who have recently carried out extended comparative studies upon this group of organisms, make no statements regarding reduction of nitrates.

Production of Gas.—None of the cultures produced gas in 2 per cent glucose broth in the fermentation tube.

* Cf. e. g. Růžička, *Arch. f. Hyg.*, 1899, xxxiv, p. 162, and Lartigau, *Journal of Experimental Medicine*, 1898, iii, p. 604.

† *Centralbl. f. Bakt.*, II. Abth., 1897, iii, p. 504.

‡ *Annals of N. Y. Acad. of Sciences*, 1895, viii, p. 322.

§ *Op. cit.*

‖ *Op. cit.*

Pigment Production.—It is in connection with the production of pigment that the most interesting, and, in some respects, the most significant differences among my seven cultures are to be noted. One culture (B. pyocyaneus, Rush) produced only one pigment, the typical, unmistakable pyocyanin; the other six, with the exception of B. pyocyaneus γ, produced both pyocyanin and fluorescent pigment; the variety labelled B. pyocyaneus γ, when it first came into my possession, produced only the fluorescent pigment, and during the year in which I have had it under observation it has lost the power of forming any pigment at all, and none of the efforts I have made to restore this power by growing the organism in media known to be favorable to the fluorescigenic property has yet proved successful.

I have made a rather detailed study of the conditions under which the pigments are produced.

Fluorescent Pigment.—The general conditions that permit the formation of the blue-green fluorescent pigment are exactly similar to those that I have elsewhere described* as necessary to the manifestation of this property by other bacteria of the same group. The presence of both phosphate and sulfate is essential; the associated cation is a matter of indifference—sodium, potassium or magnesium being equally available. Asparagin, ammonium succinate, ammonium lactate and ammonium citrate all proved suitable for the development of the fluorescent pigment.† In ammonium urate solution (amm. urate, 0.05 per cent) a small amount of pigment was produced by the cultures designated as B. pyocyaneus α and B. pyocyaneus, Mich., but the others gave only the merest trace of color. In ammonium acetate solution B. pyocyaneus, Albany, B. pyocyaneus, Mich., B. pyocyaneus α, B. pyocyaneus β and B. pyocyaneus pericard. produced pigment abundantly. Solutions of ammonium tartrate, oxalate and formate proved entirely unfavorable for the production of the fluorescent pigment, none of the species employed being able to form any pigment whatsoever in media con-

* *Botanical Gazette,* xxvii, Jan., 1899, p. 19.

† The standard solution used contained in each case 0.5 per cent of the ammonium salt and 0.1 per cent each of neutral sodium phosphate and magnesium sulfate.

structed from these salts. In the ammonium tartrate solution, abundant growth occurred in the culture of B. pyocyaneus pericard. and B. pyocyaneus, Albany, and a somewhat less abundant growth in the culture of B. pyocyaneus, Mich. In no case, however, was any pigment formed. To put the whole matter concisely, *the study of five varieties of B. pyocyaneus which produce fluorescent pigment in suitable media has entirely confirmed the conclusions arrived at in my previous work upon the production of fluorescent pigment by bacteria.**

Pyocyanin.—In spite of the inability of Thumm† during his extended study of pigment production to discover any evidence for the existence of pyocyanin, there is no doubt whatever that a blue pigment with definite optical and chemical characters is formed by some bacteria belonging to the same group of organisms that Thumm studied. The cause of Thumm's failure will be made clear presently. All the cultures that I have worked with, excepting the undoubtedly degenerate B. pyocyaneus γ, have formed pyocyanin, although in varying degrees and under different conditions. The blue pigment termed pyocyanin can be readily distinguished from the blue-green fluorescent pigment. It is soluble in chloroform while the fluorescent pigment is not; viewed by artificial light, pyocyanin is as brilliant and distinct as by daylight, while the fluorescent pigment loses its vivid emerald green tint altogether and appears a dim muddy yellow. Either of these tests for the detection of pyocyanin in the presence of the fluorescent pigment can be applied very simply. A small amount of chloroform shaken up with a fluid culture becomes, within a few seconds, a deep blue if pyocyanin be present. If cultures when viewed by daylight appear to contain nothing but the fluorescent pigment—as under certain conditions was the case with B. pyocyaneus α and B. pyocyaneus, Albany—pyocyanin can be easily detected by viewing the cultures under gaslight, when any pyocyanin present will be plainly seen owing to the blotting out of the fluorescent substance that in daylight effectually screens the other pigment.

* *Botanical Gazette*, Jan., 1899.

† *Op. cit.*, p. 367.

Another well-known difference between the pigments may also be mentioned here. Pyocyanin turns red when treated with acid, while the fluorescent substance becomes colorless, both pigments regaining their original hue when the solution is again rendered alkaline. In all the cultures that I have worked with, pyocyanin is produced more slowly than the fluorescent pigment. B. pyocyaneus, Rush, develops pyocyanin at the room temperature only after three to four days' growth, while the races that produce the fluorescent pigment at all vigorously show color 24-48 hours earlier.

One of the races that I have used for experiment, B. pyocyaneus, Rush, does not produce the fluorescent pigment, and for this reason has been particularly serviceable for the study of the conditions under which pyocyanin is produced. In ammonium succinate or asparagin solutions, prepared according to the formulæ given above, pyocyanin is produced abundantly, and I have found that after ten transfers in ammonium succinate, made at intervals of 6-8 days, the power of this race to form pyocyanin persists unimpaired, and at the end is manifested as energetically as at the beginning. This race also produces pyocyanin in lactate, acetate, and citrate solutions. A very faint blue tinge, due to pyocyanin, appears in the urate, but in the solutions of tartrate, oxalate and formate no pigment at all is formed, although a very good growth occurs in both tartrate and oxalate media. Pyocyanin is also formed by this race in beef broth and upon ordinary nutrient gelatin and agar.

Upon testing the behavior of the other varieties in the several media interesting differences came to light. B. pyocyaneus, Mich., and B. pyocyaneus, Albany, produced both pyocyanin and fluorescent pigment in succinate, lactate, acetate and citrate solutions, while B. pyocyaneus α, B. pyocyaneus β and B. pyocyaneus pericard. produced only the fluorescent pigment in these media, and did not develop any pyocyanin even after 30-40 days' growth. In the urate solution a small amount of fluorescent pigment, but no pyocyanin, was formed by B. pyocyaneus, Mich., and B. pyocyaneus α; B. pyocyaneus β did not form any pigment although rendering the solution turbid. Neither pyocyanin nor fluorescent pigment was formed by these four races in tartrate, citrate or formate media.

In standard beef broth B. pyocyaneus, Mich., and B. pyocyaneus, Albany, produced pyocyanin scantily, and the other three races formed only a small amount of fluorescent pigment. In broth from which the muscle sugar has been removed by Smith's method of inoculation with B. coli the fluorescent pigment is produced much more abundantly, but I have not observed so great a difference in the formation of pyocyanin. B. pyocyaneus, Rush, produces some pyocyanin in broth to which 2 per cent of glucose has been added, but the pigment appears more slowly than in Smith broth and the culture assumes a yellow tinge. B. pyocyaneus, Mich., also produced some pyocyanin in a solution composed of 0.2 per cent asparagin; 0.2 per cent glucose; and 1 per cent potassium sulfate. Nicolle and Zia Bey* note that their cultures produced the blue pigment in "milieux sucrés."

In nutrient gelatin all five cultures produced both pyocyanin and the fluorescent pigment; this is the only medium I have yet found in which B. pyocyaneus α, B. pyocyaneus β and B. pyocyaneus pericard. form pyocyanin.

Further experiments have been made with the three races capable of forming pyocyanin in non-proteid media with a view of determining the chemical elements essential to this activity.† The conditions are distinctly different from those governing the production of the fluorescent substance. Neither sulfate nor phosphate is essential. In a nutrient solution composed of asparagin, 0.2 per cent; magnesium sulfate, 0.1 per cent; sodium chloride, 0.5 per cent, B. pyocyaneus, Rush, produced pigment almost as abundantly and rapidly as in a standard sulfate-phosphate solution, and the pyocyanigenic power was not perceptibly weakened during four transfers in the same medium. The same thing proved true of B. pyocyaneus, Mich. If only one salt, as sulfate alone or phosphate alone, was used with the asparagin some pyocyanin was formed, but the amount in such cases was much less than under conditions more favorable to the multi-

* *Ann. de l'Inst. Pasteur*, 1896, x, p. 670.

† The cultures were kept at the room temperature unless otherwise indicated.

plication of the organism, although even in a pure 1 per cent asparagin solution some pyocyanin is formed by the three races named. It is entirely clear, therefore, that the pyocyanigenic property is in a sense more intimately bound up with the life processes of these organisms than is the fluorescigenic, since it is less dependent upon the presence of definite chemical compounds in the culture medium. Gessard* has also noticed the production of pyocyanin in a medium to which no phosphate was added, but is inclined to attribute it to the presence of small traces of phosphate carried over in the first sowing. This is of course possible, but the amount of phosphate in such a solution must be exceedingly small and the pyocyanigenic power of the organism certainly shows no such abject dependence upon the presence of this salt as is manifested in the case of the fluorescent pigment. In a medium containing 0.2 per cent asparagin, 0.1 per cent magnesium sulfate, and 0.001 per cent of neutral sodium phosphate B. pyocyaneus, Mich., produces both pyocyanin and fluorescent pigment. If the phosphate be altogether omitted pyocyanin alone is formed, and continues to be formed for at least four generations of successive transfers, which is as far as I have followed it in this species. The medium is prepared from recrystallized C. P. salts as free from phosphate as possible, and does not respond to the delicate phosphomolybdate test. Thumm† refers to Gessard's statement regarding the appearance of a blue color in the absence of phosphate and asserts that he has observed the same phenomenon in cultures of all the fluorescent bacteria that he has studied and explains the effect in this way: "Diese Färbungen sind jedoch nie auf Pyocyaninbildung sondern einfach auf Lichtbrechungserscheinungen zurückzuführen, indem jede leicht getrübte Flüssigkeit einen blauen Schimmer zeigt." Such an explanation in respect to the effects that I have observed is totally inadmissible, since I have demonstrated the presence of pyocyanin by all the applicable tests. Thumm's failure to obtain any pyocyanin here and in his other experiments with non-proteid media undoubtedly lay in the fact that the races with which he worked, whatever might have

* *Ann. de l'Inst. Pasteur*, 1892, vi, p. 810.
† *Op. cit.*, p. 367.

been their earlier history, at the time of his investigation were unable to produce pyocyanin in media constructed from ammonium salts. Three of the races in my possession show this same incapacity and the fact should be noted that they, like the races employed by Thumm, had been for some time under artificial cultivation and were perhaps obtained from the same laboratory. One of Thumm's* races seems to have behaved exactly like my own culture of "B. pyocyaneus γ" in losing its power to produce even the fluorescent pigment after it came into his possession.

Gessard† is inclined to lay great stress upon the production of pyocyanin in very simple media. "La fonction essentielle du bacille pyocyanique est donc la fonction pyocyanogène. . . . . . C'est à bon droit qu'elle entre dans son nom spécifique. C'est celle dont ne le séparent pas même ces conditions précaires d'existence, cette véritable misère physiologique qui aboutit à la mort, après un petit nombre de générations dans le milieu dépourvu de phosphate. L'autre fonction est surajoutée; elle n'est pas essentielle." I see no reason for establishing so sharp a distinction between the pyocyanigenic and the fluorescigenic function. It can hardly be doubted that cultures like B. pyocyaneus α and B. pyocyaneus β, which at present produce fluorescent pigment but no pyocyanin in ammonium succinate solutions, are merely modified descendants of races which when first isolated were more vigorous producers of pyocyanin. In fact there can be no objection to the view that the cultures of "B. pyocyaneus" that are not uncommonly found in laboratory collections and that secrete only the fluorescent pigment are nothing but degenerate offspring, so to speak, of a parent stock that originally produced both pigments. Such evidence as we possess is distinctly in favor of the view that the pyocyanigenic property is sometimes spontaneously lost while the fluorescigenic persists. The former "function" can hardly be looked upon, then, as more "essential" than the latter.

Cultural Modifications.—Gessard‡ was among the first to call atten-

* *Op. cit.*, p. 341.

† *Ann. de l'Inst. Pasteur*, vi, pp. 811, 812.

‡ *Ann. de l'Inst. Pasteur*, 1891, v, p. 65.

tion to the possibility of manufacturing new races of B. pyocyaneus by subjecting cultures to different conditions. According to this author the "typical" race, yielding both pyocyanin and fluorescent pigment, could be converted into a race possessed only of the fluorescigenic property either by the action of heat (five minutes' exposure to 57° C.) or by passage through the body of a rabbit; a pyocyanigenic race, on the other hand, could be created by growth of the typical race for a series of generations upon egg-albumen; while by the action of heat, by passage through the body of a rabbit or by spontaneous degeneration the pyocyanigenic race could be changed into a race incapable of producing any pigment; by the action of heat, too, the fluorescigenic race could be converted into a non-pigmented variety.

Acting upon these statements I have attempted to abolish or modify the pyocyanigenic power of B. pyocyaneus, Rush, by several methods. One of the first to be carried out was the passage through successive cultures in media particularly favorable to the production of fluorescent pigment, with the aim of developing some latent fluorescigenic power, but, as I have stated above, a series of ten generations in a standard succinate-sulfate-phosphate solution did not perceptibly affect the amount of pyocyanin produced and there was not the slightest approximation towards a fluorescigenic variety; on the other hand, B. pyocyaneus α, when grown in the same sulfate-phosphate medium, continued to produce only the fluorescent pigment after a parallel series of transfers. In ammonium tartrate solution no pigment is produced by either variety during the course of five generations, but on transfer to ammonium succinate the pigment characteristic of each reappears in the first generation.

Following the method suggested by Gessard, I subjected a fluid culture of B. pyocyaneus, Rush, to a temperature of 57° C. for 5 minutes, but this procedure failed to destroy the pyocyanigenic power, although development was slightly retarded in the subculture. Nicolle and Zia Bey* obtained a result similar to my own when they applied the heat method to some recently isolated cultures of

* *Ann. de l'Inst. Pasteur*, x, p. 669.

B. pyocyaneus, but Jakowski* has corroborated Gessard's statement, that the pyocyanigenic power is abolished in a culture warmed for 5 minutes at 57° C. Different races possibly differ in this regard. Neither Jakowski nor Nicolle and Zia Bey on the other hand observed any loss of pyocyanigenic power when the organism was passed through the body of rabbits. My own experiments with animals were made with mice and guinea-pigs, and are hence not strictly comparable with Gessard's, but in these animals at least no alteration of the chromogenic function could be observed in cultures isolated after death from the heart's blood, the kidneys, spleen or liver. Kukula, as quoted by Růžička,† observed sometimes exaltation, sometimes attenuation of the chromogenic power when B. pyocyaneus was passed through the animal body.

A particular interest attaches to modification experiments in view of Růžička's recent comparative studies‡ upon cultures of "B. pyocyaneus" and "B. fluorescens liquefaciens." The close relationship existing between cultures of organisms bearing these names has long been recognized. Intermediate forms are common, and no hard and fast line can be drawn between the "varieties" of the two "species." The ability to grow and produce pigment at 37° C. is perhaps as distinctive of B. pyocyaneus as any single physiological character, but the well-known ability of bacteria to adapt themselves to varying temperatures and the actual existence of a whole series of "liquefying fluorescent bacteria" that vary in behavior to temperature under natural conditions do not permit us to lay much stress upon this point of difference. Růžička attaches some importance to a freer growth of B. pyocyaneus beneath the surface of gelatin or agar media, but as I have stated elsewhere in this paper, I have not found a uniform behavior in this regard among the various pyocyanin-producing cultures in my possession. As respects indol-formation, nitrate reduction, action upon milk and growth in the ordinary nutrient media, I share Růžička's opinion that almost all characters and combinations of characters can be found among members of this group of organ-

* *Zeitschr. f. Hyg.*, xv, p. 474.
† *Arch. f. Hyg.*, 1899, xxxiv, p. 173.
‡ *Op. cit.*, p. 149.

isms. One culture will form indol, grow at 37° C. and reduce nitrate; another will not form indol but will agree with the first in all other characters, and so on through a long series in which all possible combinations of physiological capacities seem to be represented among the cells of one or another culture.

Růžička does not, however, appear to grasp the full significance of the difference between "B. pyocyaneus" and "B. fluorescens liquefaciens" in the matter of pigment production. Gessard's experiments consisted simply in removing the power to form one or the other pigment from an organism originally capable of forming both pigments. I have not been able to find in the literature a single satisfactory instance of the acquisition of pyocyanigenic power by an organism primarily devoid of this property. Kruse and Pasquale* mention the discovery of an organism resembling B. pyocyaneus except in ability to produce pigment, but do not affirm that it acquired chromogenic power on cultivation. Remlinger† records the occurrence of a non-chromogenic variety in splenic pulp and states that color appeared after the sixth or seventh sowing in broth, but I have not been able to satisfy myself that this statement necessarily implies acquisition of pyocyanigenic power. Růžička‡ describes an experiment upon the transformation of one variety into another in the course of which he noticed the formation of a "gesättigt blaugrünen Farbstoff" in a typical culture of B. fluorescens liquefaciens, but his description is not sufficiently detailed to carry conviction upon the point at issue.

I have made a number of experiments designed to augment the pyocyanigenic power of my cultures and to evoke the power in cultures in which it was latent, but thus far with only indifferent success. The pyocyanigenic power of B. pyocyaneus, Rush. was very slightly but perceptibly increased by passage through a series of cultures in ordinary peptone broth and the pigment was produced more rapidly and abundantly at the end of eight transfers than at the beginning. Similar attempts to increase the amount of pyocyanin formed by B.

* *Zeitschr. f. Hyg.*, xvi, p. 63.
† *Arch. de. méd. exp.*, 1898, x, p. 167.
‡ *Op. cit.*, p. 173.

pyocyaneus, Albany, and B. pyocyaneus, Mich., did not prove successful, and no pyocyanin at all was produced by B. pyocyaneus pericard. during the course of fifteen generations. A series of sowings in nutrient gelatin did not exalt in any degree the pyocyanigenic power in the three organisms that manifested it only in this medium. In this respect there is an agreement with what is noticed concerning the indolfacient power of the organisms.

There is, as might be expected, a difference in chromogenic power among the cells of one and the same culture. B. pyocyaneus, Mich., for example, was plated from a pure culture obtained from a single colony in the usual way. From nine separate colonies selected at random from the gelatin plate nine separate tubes of standard ammonium succinate solution were inoculated. Fluorescent pigment made its appearance in one tube on the fourth day after inoculation, in five more on the fifth day, in two more not until the eleventh day and in the ninth tube no pigment appeared until the fifteenth day. After twenty-four days the cultures were tested for pyocyanin and eight tubes showed the presence of this pigment accompanying the fluorescent. The ninth tube (one of those in which fluorescent pigment appeared on the fifth day) contained no pyocyanin, and further experiment showed that the descendants of this cell were devoid of pyocyanigenic power.

Other Pigments.—The production of other pigments besides the blue and the blue-green has been mentioned by some authors. Charrin and de Nittis* observed the simultaneous formation of black, blue, green and yellow pigments in one and the same culture. The yellow or yellow-brown pigment is, as I have shown elsewhere, an oxidation product of the green fluorescent pigment and is commonly seen in old cultures and among races beginning to lose fluorescigenic power. The black pigment has been recently attributed by Gessard† to the oxidation of tyrosin. I have observed the formation of the black pigment in old agar and gelatin cultures of B. pyocyaneus, Rush, B. pyocyaneus, Mich., and B. pyocyaneus, Albany; in other words, in the races that produce pyocyanin most vigorously. B. pyocyaneus,

* *Compt. rend. Soc. de biol.*, 1898, p. 721.

† *Compt. rend. Soc. de biol.*, 1898, p. 1033.

Rush, which is by far the best producer of pyocyanin that has come into my hands, develops the black pigment most profusely. To test the relation of this pigment to tyrosin, I inoculated a solution of 0.05 per cent tyrosin (Merck), 0.1 per cent neutral sodium phosphate and 0.1 per cent magnesium sulfate with the several cultures. Growth occurred with all, but not quite so abundantly as in the ammonium succinate medium. B. pyocyaneus, Rush, formed pyocyanin quite abundantly but rather slowly, the first appearance of the pigment being noticed in 12 days; B. pyocyaneus, Mich., and B. pyocyaneus, Albany, formed both pyocyanin and fluorescent pigment, but in scanty amounts. The chromogenic behavior of these organisms in a tyrosin medium does not, therefore, differ materially from their behavior in an ammonium succinate solution. The culture of B. pyocyaneus, Rush, in tyrosin, *which was kept in the dark*, contained only pyocyanin and had no trace of black pigment at the end of 113 days. The black pigment is, in fact, due to the oxidation of the pyocyanin. Exposure of a pyocyanin solution (an old ammonium succinate culture of B. pyocyaneus, Rush) to the action of strong north light causes a change, first to a bottle-green hue, then to a brownish-black; the same change goes on in the dark in some media, but very much more slowly than in tubes exposed to daylight. This change is one of oxidation, as is shown by the fact that a dilute solution of potassium permanganate added gradually to a pyocyanin solution produces exactly the same conversion of the blue into the bottle-green and then into the black pigment, as is wrought more slowly by the action of light and air.* I have elsewhere shown† that the conversion of the fluorescent pigment into the yellow-brown depends upon a similar oxidizing process. The formation of four pigments, then, can be easily explained on the basis of the oxidation of the blue and the fluorescent pigments into black and yellow respectively. The almost uniform occurrence of a yellow-brown tint in old stock cultures of B. pyocyaneus that have lost their ability to form either pyocyanin or fluorescent pigment may be most plausibly referred, perhaps, to an accentuation of oxi-

* Charrin and de Nittis, *loc. cit.*, state that the black pigment is produced at the surface of the agar.

† *Botanical Gazette*, 1899, xxvii, p. 19.

dizing processes, and the real explanation of the loss of pyocyanigenic and fluorescigenic power may perhaps lie in the impetus given to metabolic processes of oxidation by the conditions of life in our artificial peptone media. It must not be forgotten that both pyocyanin and the fluorescent pigment are themselves oxidation products of colorless substances.

Natural Varieties.—The confusion into which the study of B. pyocyaneus and its varieties has fallen is in part due to the rapid degeneration of many stock cultures under conditions of artificial cultivation and the consequent disappearance of all chromogenic power, and in part also to the occurrence of natural varieties characterized by considerable physiological divergence. The most common natural variety, to judge from an examination of the literature, as well as from the study of a number of freshly isolated cultures that I have obtained from different sources, is the one that produces both pyocyanin and the fluorescent pigment. This is the variety that was originally described and studied by Gessard, and I would suggest that the name B. pyocyaneus, var. α be given to freshly isolated cultures possessed of this double chromogenic power. The culture with which Ernst worked* and to which he gave the designation of "α," was, so far as I can determine from his description, a culture wholly devoid of pyocyanigenic power and one that produced, while in his hands, only the fluorescent pigment.† The most natural inference is that in respect to pyocyanin production this culture was degenerate. Many cultures seem overtaken by this fate. It is perhaps fair to assume that the large number of cultures found in laboratory collections bearing the name of B. pyocyaneus, but without any power to produce pyocyanin, are degraded scions of a more vigorous parent stock.‡ It is worthy of note that the pyocyanigenic property is apparently the first to be lost. I have never observed a case where the fluorescigenic

* *Zeitschr. f. Hyg.*, ii, p. 369.

† Cf. e. g. the statement on p. 373, *op. cit.*, that the color of cultures of the α-bacillus disappears when viewed by gaslight.

‡ The failure to preserve unimpaired the original pyocyanigenic power is clearly shown in the usual inability of old cultures to manifest the "chameleon phenomenon," while most freshly isolated cultures display this power (cf., for example, Schürmayer (cited below) and Lartigau (*loc. cit.*).

power was lost by spontaneous degeneration while the pyocyanigenic persisted, nor have I found mention of such a case in the literature.

It is a matter of common experience that there is great diversity among cultures of B. pyocyaneus as regards rapidity of degeneration. Some lose the power to produce either pyocyanin or fluorescent pigment within a short time after isolation; others weaken quickly in pyocyanigenic power, but retain the fluorescigenic much longer; others again, but these are the rarer cases, show almost undiminished capacity for the formation of pyocyanin and fluorescent pigment through a long series of generations. B. pyocyaneus, Mich., is the most notable instance of the last group with which I am acquainted and is a marvelously stable variety; it has shown no attenuation in chromogenic power during the three years and a half I have had it under observation.

In the relative proportion of pyocyanin and fluorescent pigment there is likewise considerable variation among freshly isolated cultures. New varieties have even been founded by some authors upon the differing nuances of color due to varying admixtures of the two pigments, but the advisability of such distinctions is questionable. It must be acknowledged that there are found races of the variety that I have termed var. α, some of which are more or less sharply distinguished from one another by intensity and persistency of pigment production as well as by such physiological traits as the formation of indol, reduction of nitrates and growth on potato, but it must be left to future bacteriological investigation to interpret the significance of these deviations and to determine to what extent subvarieties and races shall be established.

The culture I have designated as B. pyocyaneus, Rush, produces only pyocyanin under ordinary conditions of cultivation, and has resisted all my attempts to communicate to it any fluorescigenic power. I would suggest that the name B. pyocyaneus, var. β, be employed to distinguish this non-fluorescigenic variety. I am unable to determine whether the variety studied by Ernst,* and designated by him as the "β variety," produced only pyocyanin, but the coloring of his

* *Zeitschr. f. Hyg.*, ii, p. 369.

figures (*op. cit.*, p. 372), and the peculiar liquefaction of gelatin there shown, together with the statement regarding tardy appearance of the color, suggest that this may have been the case. Schürmayer* and Jakowski† both worked with cultures of the pyocyanigenic-fluorescigenic variety, which I have called the " α " variety. Nicolle and Zia Bey‡ also appear to have been dealing with the α-variety, although they state that their four cultures were " much more pyocyanigenic than the type." I have not been able, in fact, to find in the literature any unimpeachable statement of the existence of a purely pyocyanigenic variety such as I have worked with, although it is not difficult to discover instances where the pyocyanigenic power of a culture has been recognized to be much higher than is usually the case.

The occurrence of a fluorescigenic variety seems to be more common. The variety I have termed the α-variety frequently degenerates spontaneously into this. The chief difference between this variety and the organism called B. fluorescens liquefaciens seems to lie in the difference of temperature optimum and perhaps in behavior in the animal body (Růžička). I have not been able to confirm Růžička's observations respecting a constant difference in gelatin stab-cultures. It is a tempting hypothesis that B. fluorescens liquefaciens is a degenerate or modified form of B. pyocyaneus, but more convincing evidence than we now possess is needed to establish this point.

The natural occurrence of a non-chromogenic variety has been affirmed by Remlinger§ and others. Kruse and Pasquale‖ have recorded the finding of a non-chromogenic variety exactly like the ordinary B. pyocyaneus, but without power to produce pigment. I have not been able to obtain a natural variety of this kind. It will be generally recognized that the method of repeated transfer of such a variety from one tube of fluid culture to another (Remlinger) is open to objection unless frequent control platings are carried out. My own attempts to restore chromogenic power to spontaneously or artificially etiolated cultures have failed.

* *Zeitschr. f. Hyg.*, xx, p. 281.
† *Zeitschr. f. Hyg.*, xv, p. 474.
‡ *Ann. de l'Inst. Pasteur*, p. 669.
§ *Op. cit.*
‖ *Op. cit.*

CONCLUSIONS.

The principal conclusions that seem to me justified are as follows:

1. The fluorescent pigment formed by some varieties of B. pyocyaneus is produced under conditions identical with those governing the production of the pigment by other "fluorescent bacteria."*

2. The production of pyocyanin is not dependent upon the presence of either phosphate or sulfate in the culture medium. It is formed in non-proteid as well as in proteid media, but is not a necessary accompaniment of the metabolic activities of the organism (*e. g.* tartrate solution).

3. The power of producing pyocyanin under conditions of artificial cultivation is lost sooner than the fluorescigenic power.

4. There are greater natural and acquired differences in pyocyanigenic power than in fluorescigenic.

5. The fluorescent pigment may be oxidized slowly by the action of light and air as well as by reagents into a yellow pigment, and pyocyanin may be similarly oxidized into a black pigment.

6. A convenient separation of B. pyocyaneus into four varieties would be the following: var. α, pyocyanigenic and fluorescigenic (most common); var. β, pyocyanigenic only (rare); var. γ, fluorescigenic only (not uncommon, closely related to "B. fluorescens liquefaciens"); var. δ, non-chromogenic.

7. Except for the occasional loss of one or another function the different varieties are not so plastic as sometimes assumed, and cannot be readily converted into one another by subjection to varying conditions of life.

8. The signification and correlation of the almost countless physiological variations among the members of this group in respect to growth in gelatin, behavior to temperature, indol production, etc., remain to be determined. It is not yet clear that the variations in chromogenic power can be in any way correlated with the presence or absence of other physiological functions.

* Cf. *Botanical Gazette*, xxvii, p. 19.

A PRELIMINARY NOTE ON THE FRACTIONAL PRECIPITATION OF THE GLOBULIN AND ALBUMIN OF NORMAL HORSE'S SERUM AND DIPHTHERIC ANTITOXIC SERUM, AND THE ANTITOXIC STRENGTH OF THE PRECIPITATES.

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It has been shown by investigators that the globulin of tetanus and diphtheria antitoxic sera, when separated from the sera, carries with it the antitoxic power of the sera. It has not been proved, however, that the "antitoxin" is a globulin. My work of the past two years has been directed to the study of the proteids of different antitoxic sera, especially diphtheria antitoxic serum.

An article in the *Zeitschrift für Hygiene und Infectionskrankheiten* (1889, xxxi, p. 513) by Dr. W. Seng, entitled "Ueber die qualitativen und quantitativen Verhältnisse der Eiweisskörper im Diphtherieheilserum," suggests to me that it would be well to publish a preliminary note on my work.

When the globulin, which has been separated from horse's serum by means of magnesium sulphate and purified by dissolving in water, reprecipitating and washing with a saturated magnesium sulphate solution, is dissolved in water, and this solution of purified globulin is saturated with sodium chloride, a precipitate is formed. This I will call the room temperature precipitate (R° ppc.). If, after the removal of the R° precipitate by filtration, the temperature of the filtrate be raised to 40° C., a little more salt having been added to insure saturation, a turbidity appears in the fluid and when the temperature of 44° or 45° C. is reached the precipitation is complete for this temperature.

Proceeding in the same manner one obtains a third turbidity at 49°

C. and complete precipitation at 53° C., a fourth turbidity at 57° C. and complete precipitation at 62° C., and finally a fifth turbidity at 67° C. and complete precipitation at 72° C. These precipitates dissolve in water, except a very small quantity of that obtained at 72° C., which is insoluble in water but redissolves immediately when treated with weak sodium hydroxide.

Exactly the same reactions occur when diphtheric antitoxic serum is employed instead of normal horse's serum. In both sera the final filtrates fail to give the biuret reaction, and on boiling and subsequent addition of a little acetic acid show no turbidity. Each one of the separate fractions of the antitoxic globulin possesses an antitoxic power while the final filtrate is free from antitoxin.

After the globulin precipitated by the magnesium sulphate in both the normal and diphtheric antitoxic sera has been filtered off, the albuminous filtrate is saturated with sodium chloride. One then finds that an albuminous precipitate is not formed at room temperature. A double salt which is formed by the magnesium sulphate and sodium chloride is removed by filtration and the filtrate is raised in temperature to 56° C. when a turbidity is formed. At 61° C. the precipitation is complete. At 68° C. a second turbidity appears and can be filtered off at 72° C. At 73° C. a third turbidity appears and at 76° C. this precipitation is complete. Finally at 77° C. a slight turbidity appears and at 81° C. the precipitation is complete. The final filtrates from the 81° precipitation fail to give the biuret reaction, and on boiling and subsequent addition of acetic acid show no turbidity.

The albumin precipitated at 56° C. is soluble in water. The other three precipitates are partially soluble in water, and completely so in sodium hydroxide.

In a paper which will shortly appear I shall give the quantitative relations between the corresponding fractions of the normal and antitoxic globulin and albumin, and the antitoxic value of the fractions of the antitoxic globulin.

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